Graduate School ETD Form 9
(Revised 12/07)

PURDUE UNIVERSITY
GRADUATE SCHOOL
Thesis/Dissertation Acceptance

This is to certify that the thesis/dissertation prepared

By Kelley M. Kitts

Entitled
Evaluation of Odor Compounds Sensed by Explosives-Detecting Canines

For the degree of Master of Science

Is approved by the final examining committee:

Jay Siegel
Chair

Lei Lee

John Goodpaster

To the best of my knowledge and as understood by the student in the *Research Integrity and Copyright Disclaimer (Graduate School Form 20)*, this thesis/dissertation adheres to the provisions of Purdue University's "Policy on Integrity in Research" and the use of copyrighted material.

Approved by Major Professor(s): John Goodpaster

Approved by: Christine Picard 2/9/12
Head of the Graduate Program Date

Graduate School Form 20
(Revised 9/10)

PURDUE UNIVERSITY
GRADUATE SCHOOL

Research Integrity and Copyright Disclaimer

Title of Thesis/Dissertation:

Evaluation of Odor Compounds Sensend by Explosives-Detecting Canines

For the degree of Master of Science

I certify that in the preparation of this thesis, I have observed the provisions of *Purdue University Executive Memorandum No. C-22,* September 6, 1991, *Policy on Integrity in Research.**

Further, I certify that this work is free of plagiarism and all materials appearing in this thesis/dissertation have been properly quoted and attributed.

I certify that all copyrighted material incorporated into this thesis/dissertation is in compliance with the United States' copyright law and that I have received written permission from the copyright owners for my use of their work, which is beyond the scope of the law. I agree to indemnify and save harmless Purdue University from any and all claims that may be asserted or that may arise from any copyright violation.

Kelley M. Kitts

Printed Name and Signature of Candidate

2/9/12

Date (month/day/year)

*Located at http://www.purdue.edu/policies/pages/teach_res_outreach/c_22.html

EVALUATION OF ODOR COMPOUNDS SENSED BY EXPLOSIVES-DETECTING CANINES

A Thesis

Submitted to the Faculty

of

Purdue University

by

Kelley M. Kitts

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

May 2012

Purdue University

Indianapolis, Indiana

.

I would like to dedicate this work to my fiancé, Quiggs, for his support and love throughout my educational journey. I owe my gratitude to my sister and best friend, Linde, for her prayers, guidance, and undying loyalty. Finally, to my three partners in crime Ryan, Chad, and Aaron, thank you for keeping me sane and always making me laugh.

ACKNOWLEDGEMENTS

I would like to thank John Goodpaster, Ph.D., my graduate advisor, for his guidance, support, and most importantly, patience. I would like to thank the Technical Support Working Group and the Department of Defense for financially supporting this research. Finally, I would like to thank Jay Siegel, Ph.D., and my defense committee for their time and support.

TABLE OF CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

ABSTRACT

Kitts, Kelley M. M.S., Purdue University, May 2012. Evaluation of Odor Compounds Sensed by Explosives Detecting-Canines. Major Professor: John V. Goodpaster.

Canines are regularly utilized by law enforcement agencies to detect explosives. However, the mechanism by which canines respond to explosive vapors is not well understood, leading to difficulties in canine training and testing. It is known that the amount of vapor generated from explosive compounds is dependent upon several factors including sample amount, vapor pressure, and the degree of confinement. Underlying these factors is the basic process of evaporation of an unconfined explosive, which is crucial to understanding how explosive vapors behave in other, more confined, systems. In Stage One of this study, evaporation rates were determined for several explosive liquids using an analytical balance. These rates were compared to one another as well as to theoretical models for the evaporation of liquids. In general and as expected, mass decreased linearly with time and evaporation rates decreased logarithmically as boiling point increased. Several examples of solvent "pinning" on a metal surface were also observed.

While an empirical model for the evaporation of unconfined explosive liquids was developed, a comprehensive model for the escape of explosive vapors from sealed containers (i.e., a suitcase, knapsack, or IED container itself) is needed. The second part of Stage One of this study was to determine that the flow rate of explosive vapors escaping from relatively large orifices does not conform to Fick's Law of Diffusion. Fick's model states that the flow rate is linearly dependent upon the cross sectional area of the orifice and the material's diffusion coefficient. Instead, the flow rate was found to be linearly dependent upon the diameter of the orifice due to the tendency of the flow to diffuse outwards from its circular edge. A clear relationship between flow rate and diffusion coefficient was seen, however.

Additional uncertainty arises concerning the complexity of the odor generated from explosive compounds. Because explosive vapors are often complex (they consist of multiple chemical compounds), confusion exists regarding the cause of canine alert; that is the "odor compound" that allows for canine detection of various explosives. Although 2, 4- dinitrotoluene (DNT) has been explored as a potential odor compound, the possibility of a nitrated explosive inherently producing nitrated gas upon decomposition has not. Stage Two of this study focused on evaluating nitrate as a potential cause of canine alerts. An LC/MS method for the detection of nitrate ions in Composition C-4 and flake trinitrotoluene (TNT) was developed and tested. Instrumental analysis was not successful in detecting nitrate ions in any of the explosives tested. The lack of nitrate was confirmed using a diphenylamine color test for nitrates, thus eliminating nitrate as an odor compound and cause of canine alert to nitroaromatic compounds.

2, 4-DNT has been introduced as a potential odor compound of TNT, however, the mechanisms behind its vapor emission have not been thoroughly explored. More specifically, due to the "sticky" nature of the 2, 4-DNT isomer, the effects of surface adhesion to container walls are of concern. In particular, whether the amount of material lost to surface adhesion is significant enough to effect canine detection of TNT. A second focus of Stage Two explored this concern. A GC/MS method for the detection and separation of TNT and DNT isomers in liquid extracts was developed and the amount of 2, 4-DNT residues adhering to container walls was quantified. These values, compared to the amount 2,4-DNT expected to saturate each container (determined by the Ideal Gas Law), showed a significant preference of 2,4-DNT in the solid phase as opposed to in the gas phase. The amount of residue adhering to the walls of a gallon can differed from expected values by nearly 70%. The amount of material extracted from a quart can exceeded expected values by 137%. The apparent sticky nature of 2, 4-DNT resulted in a significant loss of material needed to fully saturate a container and thus canine detection success may be affected.

In the final stage of this study, theories regarding odor compounds and odor availability of nitromethane, TNT, and Composition C-4 were tested using certified explosives-detecting canines. These trials included thirty-three canine-handler teams from eight government agencies. The odor availability of nitromethane was tested by placing varying volumes of nitromethane in containers with differing degrees of confinement and studying the effects on canine detection success. The odor availability trial showed no significant effect of sample amount or degree of confinement on canine

detection so long as the sample volume was sufficient to saturate its container. In this study that volume was determined to be < 1 mL.

Detection of 2, 4-DNT, TNT-NESST (Non-Hazardous Explosives for Security Training and Testing), and flake TNT were also studied using certified canines. The purpose of this was to identify the odorant responsible for canine alert to the explosive TNT. These trials showed a significant response to 2, 4-DNT compared to TNT and its training aid; this suggests that 2, 4-DNT is the primary cause of canine alerts to TNT. Additionally, Composition C-4 and RDX-NESTT were tested along with potential odor compounds that included the manufacturing solvent, cyclohexanone, the energetic "taggant" 2, 3-dimethyl-2.3-dinitrobutane (DMNB), the plasticizer dioctyladipate (DOA) and its degradation product 2-ethyl-1-hexanol. While some response to DMNB and cyclohexanone was seen, the most significant response was to the actual Composition C-4. This suggests that the cause of canine alert to Composition C-4 is the explosive mixture as a whole and not a single chemical component of the mixture.

CHAPTER 1. INTRODUCTION

1.1. Canine Detection

For decades, the sensitivity of a canine's olfactory system and its ability to discriminate odors has been recognized. To date, canines are regarded as one of the most effective real-time detectors of explosives, however this remarkable ability is not limited to explosive devices. Canines have been known to use their olfactory senses to hunt, mate, reproduce, and alert to danger [1]. More recently, canines have been used for the detection of narcotics, cadavers, weapons, gold ore, plant and animals species, and even illness [2]. Several studies have been conducted to explore the extent to which a canine's olfactory system can discriminate and identify volatile compounds based on the compound's physical and chemical characteristics; however, the mechanisms behind this ability are not well understood.

Examination of the canine olfactory system shows a highly developed olfactory lobe within the brain along with the presence of an additional olfactory chamber called the vomeronasal organ. Furthermore, canines possess over 220 million olfactory receptors compared to the human's 5 million [3]. It is this ultra-developed sensory system that allows canines to require only trace amounts of volatile components to achieve successful detection.

The process of canine sampling and discrimination begins with inhalation (*i.e.* sniffing), whereby vapor molecules enter the nasal cavity and become dissolved in the mucosal lining [2-4]. Once dissolved, the molecules come into contact with specialized epithelial cells that interact with olfactory sensory neurons (OSN). The OSN serve as the primary sensing cells; each neuron extends and projects through the nasal lining ending with a "knob like" swelling that projects 20-30 cilia [4]. Once an odor compound encounters these cilia, impulses are sent to the canine's olfactory lobe within the brain for scent recognition.

Beyond these basic physiological facts, little more is understood regarding the process of scent recognition and interpretation by canines. It is known that canines have the ability to recognize a single odor compound amongst a matrix of odorants with remarkable sensitivity. Attempts to mimic this discrimination and sensitivity have resulted in various forms of technology. Substitutes for odor detection include the use of ion mobility spectrometry for the detection of trace levels of nitro-organic explosives [5]. This technology has been utilized at airports and in the field. Other attempts involve development of "electronic noses" and sensors for various applications, however, none have yet to exhibit the success rate of actual canines [6-8]. It is for this reason that the Department of Defense has funded this research exploring the mechanisms behind canine detection; a better comprehension of these mechanisms will aid in the development of more effective testing and training protocols.

1.2. Odor Availability

Although the detection of explosive vapors has been well studied [9, 10], the amount of vapor available to explosive-detecting canines has not. The concept of odor availability in reference to explosive detection refers to the amount of vapor available for canines sampling. Little is understood regarding odor availability and thus several areas of uncertainty exist, particularly concerning the effect of sample amount. It has been hypothesized that the amount of explosive present is the key contributor to odor availability; in reality odor availability is affected by several factors including vapor pressure, diffusion coefficient, sample amount, environmental temperature, and the degree of confinement.

Research into the underlying factors affecting odor includes characterization of vapor pressure [11] and the effects of surface adhesion [12] of explosives. Furthermore various methods of explosives detection have been developed [9]. The problem lies with the fact that most studies are focused on developing various methods of detection, be it in the field or in a laboratory while the mechanisms of vapor movement have been greatly ignored. If canine detection and training is to be optimized, exploration of these factors must occur.

One particular area of uncertainty arises with vapor generation and transport by explosives, most notably evaporation, or the rate of mass loss. Evaporation involves both heat and mass transfer and the evaporation of an unconfined explosive must be modeled in order to understand how vapors behave in more confined systems. Once the behavior of a confined explosive is understood, exploration and understanding of the flux, that is the amount of

material transferred through an area over time [13, 14], may be evaluated. Because most explosive devices are concealed, (e.g. underground, in suitcases, car trunks, etc.) an understanding of the movement of vapors through and around obstacles must be developed.

First, the evaporative behavior of various explosive liquids will be monitored to develop an empirical model for vapor generation. Next, the flux of explosive vapors from perforated containers will be evaluated and a model describing this behavior will be introduced. Finally, these models will be tested using certified explosives-detecting canines to determine the effects of sample amount and confinement. Nitrated liquids (nitromethane, nitroethane, and nitropropane) were used in the odor availability studies because they are known components of binary high explosives, are volatile at room temperature, and are readily available in pure form making them ideal testing compounds.

1.3. Odor Compounds

In the wake of the September 11, 2001 attacks on the World Trade Center, detection of hidden explosive devices has been as the forefront of explosives research. Current methods of explosives detection include irradiation with subatomic particles, swabbing for explosive residues, sampling via high velocity air flows, and detection of volatile compounds via vapor detectors and/or canines [9]. Despite the development of artificial sensors, canines are still considered the most reliable and sensitive means of detecting of explosives and their residues [15].

This form of detection is exceptionally advantageous due to its non-invasive nature and the detection of trace levels of explosive. In spite of the success of canine detection thus far, a better understanding of explosives and the vapors they generate will aid police agencies by developing more effective canine testing and training programs.

The vapors emanating from explosive compounds are often complex, meaning their headspace consists of several chemical compounds. Thus isolation of a specific odor as the cause for canine alert is difficult. These compounds can originate from one or more species within the sample, be a degradation product of the parent explosive and/or species, or both. Even more confusing are the notoriously non-volatile explosives (e.g. TNT, RDX, PETN) whose vapor pressures are low and thus inhibit detection [9]. These compounds may generate explosive related compounds (ERCs), which are developed upon degradation of the base explosive. An example of this is 2,4-DNT, an impurity that is commonly found in samples of the explosive TNT. Additionally, explosives often contain "taggants" which are volatile energetic compounds added to plasticized explosives (such as Composition C-4) to aid in detection. In such a case, the taggant becomes a significant component of the odor generated from the explosive. Finally, some have introduced the idea of "signature compounds" which are volatile solvents, additives, or impurities within the explosive mixture, as potential odor compounds, Each of these odor compounds has been introduced as a possible cause of canine alert, specifically for those explosives which are non-volatile in nature.

This study will explore the possibility of each of these compounds as potential odor compounds. First, the possibility of nitrated explosives producing nitric acid which degrades to nitric oxides will be explored using LC/MS. If nitrates are present within the explosives then they will be detectable in aqueous extracts and will yield acidic solutions. Next, 2,4-DNT will be explored as a potential odor compound and a method for the detection and separation of 2,4-DNT and its residues within a container will be developed using GC/MS. Finally, each potential odor compound will be tested using certified explosives-detecting canines.

CHAPTER 2. EVAPORATION OF UNCONFINED EXPLOSIVE LIQUIDS: IMPLICATIONS FOR EXPLOSIVES-DETECTING CANINE TRAINING AND TESTING

2.1. Introduction

Canines are often used to detect characteristic volatile compounds emitted by explosives, but there are several areas of uncertainty regarding the mechanism by which this is achieved. For example, it is a common misconception that the amount of explosive vapor available for detection is solely dependent on the amount of explosive present. In reality, this quantity is dependent on additional factors such as the explosive vapor pressure [11], the degree to which the explosive is confined, and the extent of mass transport. It has also been shown that vapors emanating from certain nitrated explosives tend to adsorb onto surrounding surfaces, thus reducing their ability to penetrate into their surroundings [12].

Taken together, these factors can be combined into a concept known as “odor availability”, which refers to the amount of explosive vapor available for sampling by a canine. This concept remains controversial in the explosive-detecting canine community because the quantity of explosive used for canine testing and/or training is easily monitored.

The environmental conditions in which the explosives are found, however, are not. Some practical aspects of odor availability have been studied, such as the detection limit of trained canines for a volatile explosive such as nitromethane [16].

If odor availability is thought of as a process, the first step is the evaporation of volatiles from the explosive itself. In this case, evaporation is expressed as the rate with which the mass of an explosive material decreases over time. Although seemingly a simple process, evaporation is a complex phenomenon that involves both heat and mass transfer. Several theories exist that can describe evaporation under various conditions; one of the more useful models describes the evaporation of a stationary drop of liquid that conforms to a spherical-cap shape on a flat surface under still atmospheric conditions. This situation has been described by Deegan [17, 18] (see Figure 2.1) and expanded to include the effects of thermal conductivity of the substrate by Dunn, *et al* [19].

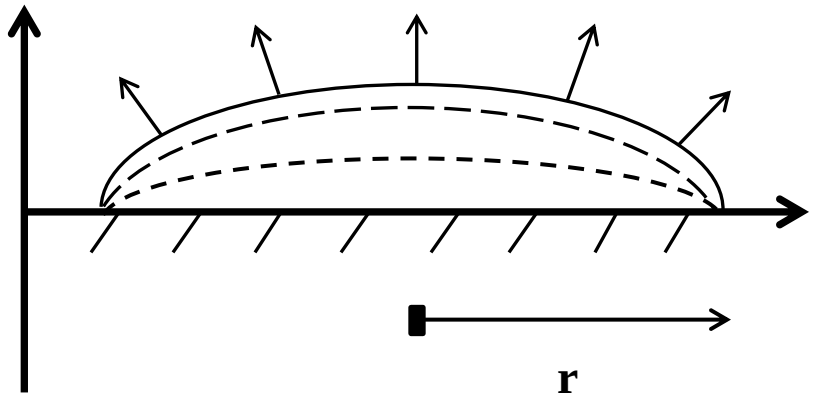


Figure 2.1: Graphical Representation of the Deegan Model, where a "pinned" liquid drop on a flat surface evaporates but the radius does not change.

Ultimately, the Deegan model results in the following equation for the total evaporation rate (in units of grams per second):

$$-\frac{dm}{dt} = 4rD(1 - H)c_{sat}(T_a) \quad \text{(Equation 2.1)}$$

where r is the radius of the droplet in cm, D is the diffusion coefficient in units of cm^2/sec, H is relative humidity (used for aqueous samples), and c_{sat} (T_a) is the standard concentration of the vapor at ambient temperature T_a in units of g/cm^3. In the original work by Deegan, drops containing colloidal suspensions were observed to "self-pin" so that r was constant during the initial phase of the drying process. This results in a linear decrease of mass with time. Eventually, however, the droplets "de-pinned" and began to shrink, whereupon the mass loss reverts to being dependent upon the drop radius.

This type of model is relevant to liquid explosives, whose unconfined evaporation must first be understood in order to fully account for their odor availability in more confined environments. The goal of this study is to use the Deegan theory to describe the evaporation of several nitrated liquids that either explosives themselves or are found in explosive formulations.

2.2 Materials and Methods

Six nitrated liquids were used in this study: nitromethane (Sigma Aldrich, St. Louis, MO), nitroethane (Sigma Aldrich, St. Louis, MO), 1-nitropropane (Sigma Aldrich, St. Louis, MO), nitrobenzene (Fisher Scientific, Fair Lawn, NJ), 2-nitrotoluene (Alfa Aesar, Ward Hill, MA), and 3-nitrotoluene (Sigma Aldrich, St. Louis, MO). Varying volumes of these liquids were placed in either glass watch glasses or sample containers employed in the National Odor Recognition Test (NORT) for canine testing [20]. The latter were 2 ounce "sniffer tins" that are typically used to hold a small sample of explosive. Sniffer tins consist of a two ounce metal container with a perforated lid and the typical diameter of the holes is on the order of 1 mm with a total area of ~ 12 mm^2 [16, 21]. In all cases, evaporation occurred with the sniffer tin or watch glass unconfined and open to the atmosphere. A video clip showing the behavior of the fog generated by dry ice when it is placed inside an unconfined sniffer tin is available as supplemental material.

Mass loss measurements were obtained in triplicate using an accuSeries accu-124 (Denver Instruments, Denver, CO) digital analytical balance. The accu-124 balance was connected via USB connection to a Dell computer running Pinnacle USB software. The mass loss was measured in two second intervals and data was logged into a Microsoft Excel 2007 workbook. The raw data was reported as milligrams of liquid lost per second. From here a plot of sample amount (in milligrams) versus time (in seconds) was created. Linear regression was then performed for data points ranging from 0 to 30 minutes and the slope of the resultant line was reported as the evaporation rate ($\frac{dm}{dt}$).

Pictures of the evaporation of the liquids were acquired with a Cannon Rebel T1i with Cannon EOS Utility. In order to obtain the area of the liquid pool, the internal diameter of the watch glass or sniffer tin was used to calibrate an image analysis software package (ProAnalyst). The image of the pool was outlined manually with a white filled polyline and the brightness was turned completely down leaving only a bright white pool against a black background. Contour tracking was then used to determine the area of the pool and the results were exported to Excel.

All diffusion coefficients were calculated using the Environmental Protection Agency (EPA) diffusion coefficient calculator available at their website [22]. The diffusion coefficient calculator uses the Fuller, Schettler, Giddings (FSG) method developed in 1966 to predict binary gas phase diffusion [20]. Diffusion coefficients were estimated at room temperature (298 K) at 1 atm.

2.3. Results and Discussion

The physical and chemical properties of the liquids used in this study are listed in Table 2.1. These liquids are found in binary explosive formulations or as volatile taggants that are deliberately incorporated into plastic bonded explosives. A binary explosive consists of a fuel and oxidizer that do not become explosive until mixed. Because of this, the two components can be shipped separately and without special handling requirements. The use of volatile taggants in explosives was devised due to the difficulty in detecting many high explosives. In this case, a volatile taggant is defined as a chemical substance with a high vapor pressure that is deliberately added to an explosives formulation to enhance its detectability.

Table 2.1: Chemical and Physical Properties for Various Nitrated Explosives.

Compound	Molar Mass (g/mol)	Boiling Point (K)	Vapor Pressure @ 298 K (Pa)[23]	C_{sat} @ 298 K (mg/cm^3)	Diffusion Coefficient @ 298 K (cm^2/sec)[a]	Application
Nitromethane	61.04	374	4790	1.18×10^{-1}	0.122	Binary high explosive
Nitroethane	75.07	387	2790	8.45×10^{-2}	0.103	Binary high explosive
Nitropropane	89.09	405	1360	4.89×10^{-2}	0.091	Binary high explosive
Nitrobenzene	123.12	484	30	1.49×10^{-3}	0.077	Binary high explosive
2-Nitrotoluene	137.14	495	12.7[23]	7.03×10^{-4}	0.071	Taggant
3-Nitrotoluene	137.14	505	8.55[23]	4.73×10^{-4}	0.071	Taggant

[a] Estimated using EPA Online tools for site Assessment Calculation (www.epa.gov)

Mass loss measurements were taken to compare evaporation of 1 mL quantities of nitromethane from both confined and unconfined sniffer tins. These measurements showed that the free evaporation of nitromethane in open air proceeds rapidly, approximately 4.8 times more rapidly than evaporation from a confined container (see Figure 2.2). It was also noted that while confined evaporation conformed to a linear regression, the unconfined evaporation showed better conformity to a quadratic function. This would agree with Deegan's theory which states that mass loss over time is initially linear due a constant radius, however upon de-pinning, mass loss then reverts to a quadratic relationship between mass loss and time.

It was also noted that the evaporation rate of nitromethane in open air increased with increasing sample amount. This increase is ascribed to the increased surface area of the larger volume samples. Under constant flux conditions, this results in a larger flow rate of material from the sample. Note that the evaporation rate of the 10 mL sample was less than would be expected at this volume; this is because the volume of liquid filled the bottom of the sniffer tin, and was thus confined to an area less than would naturally occur if the pool was allowed to spread over a flat surface (see Figure 2.3).

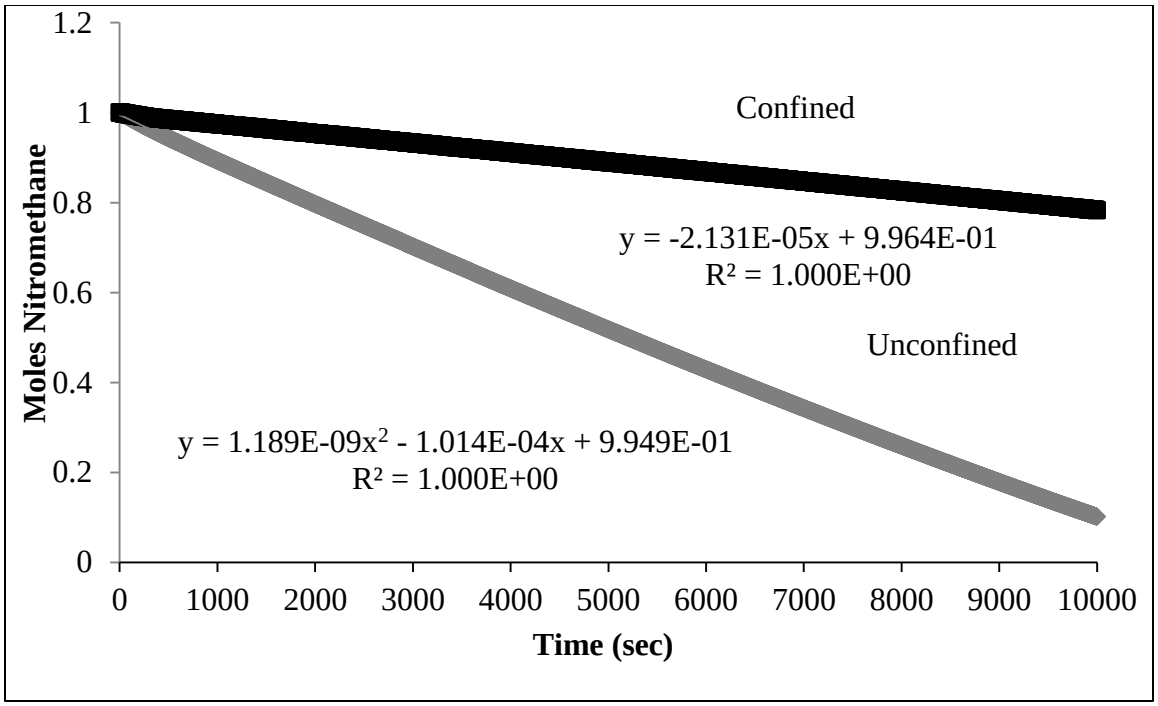


Figure 2.2: Unconfined versus Confined Evaporation of 1 mL of Nitromethane

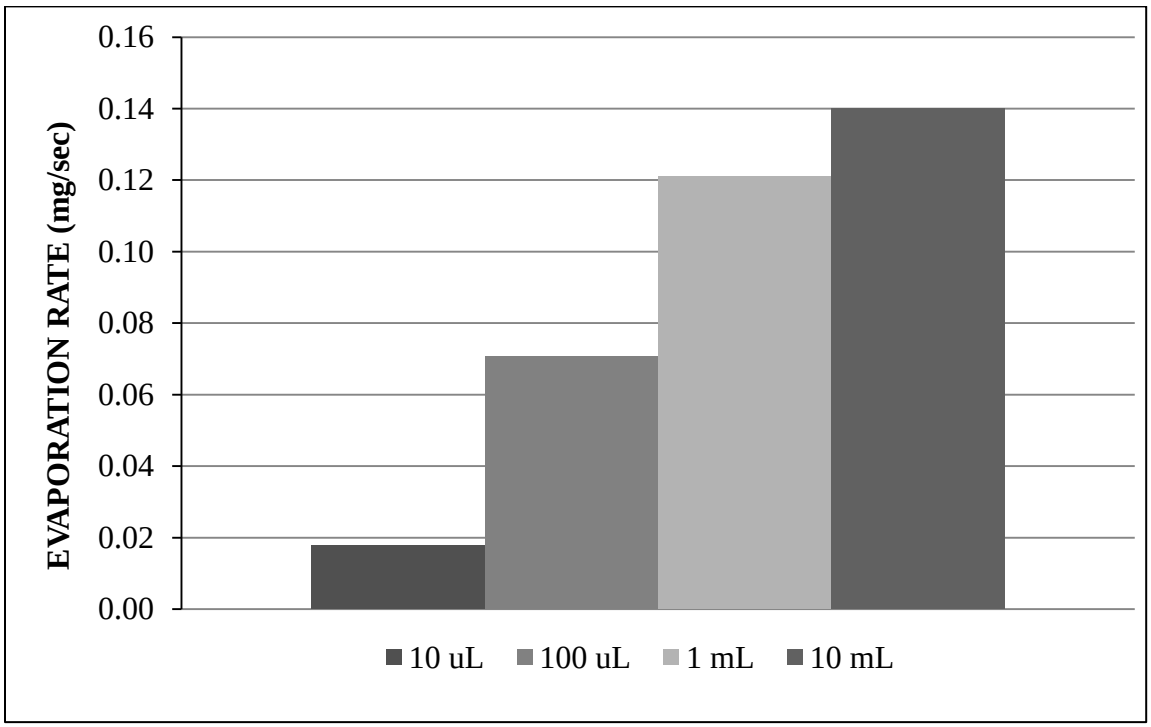


Figure 2.3: Mass Loss of Varying Amounts of Nitromethane in Open Air. Evaporation rates were collected for the initial time period during which 10% of the sample mass was lost.

The rate of evaporation was markedly lower for the other nitroalkanes. For example, the molecular weight of the nitroalkane directly affected evaporation rates as heavier compounds such as nitroethane and nitropropane evaporated more slowly than the lighter nitromethane (see Figure 2.4). Because evaporation involves mass transfer one would expect such behavior. Table 2.2 contains the measured evaporation data for all six liquids, where the mass was monitored for 30 minutes and the slope of the mass loss curve was fit to a line.

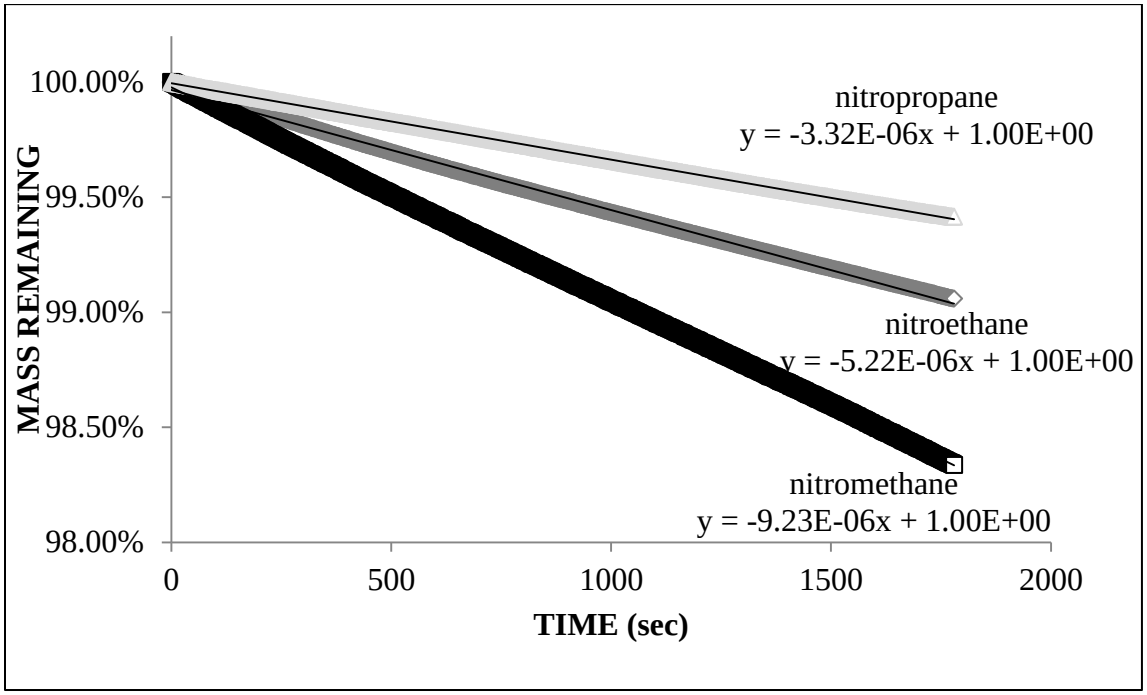


Figure 2.4: Mass Loss Curves for Nitroalkanes. Using 1 mL quantities of nitromethane, nitroethane, and nitropropane on a flat metal surface in open air at short times.

Table 2.2: Summary of Measured Evaporation Rates for Unconfined Nitrated Liquids.

Compound	Boiling Point (K)	Measured Evaporation Rate (mg/sec)		
Nitromethane	374	0.132	±	0.013
Nitroethane	387	0.0779	±	0.0032
Nitropropane	405	0.0466	±	0.0037
Nitrobenzene	484	0.00355	±	0.00033
2-nitrotoluene	495	0.00208	±	0.00067
3-nitrotoluene	504	0.00109	±	0.00007

A distinction between liquids of different chemical classes was also clear; in particular, the unconfined evaporation of nitroalkanes proceeded more rapidly than their nitroaromatic counterparts. A semi-logarithmic graph of the measured evaporation rates as a function of boiling point is included in Figure 2.5. The data is well-fit by an exponential function, which is appropriate given the exponential relationship between temperature, enthalpy of vaporization and vapor pressure as expressed in the Clausius-Clapyron equation.

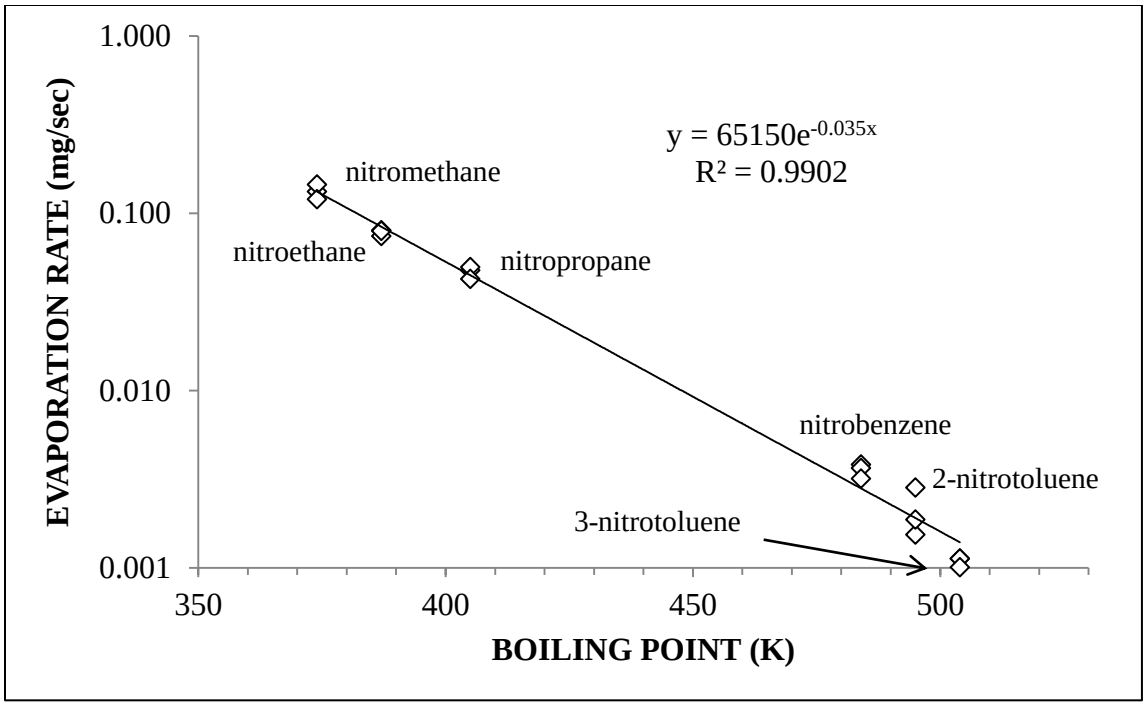


Figure 2.5: Evaporation Rate versus Boiling Point of Nitroalkanes.

The evaporation of nitromethane also demonstrated behavior as discussed by the Deegan Model. For example, these results show that for 1 mL samples evaporating on a flat metal surface over short times (*i.e.*, less than 2 hours), mass was a linear function of time, and area remained largely constant (see Figures 2.6 and 2.7). This corresponds to a "pinned" condition in the Deegan Model where the boundaries of the pool did not change appreciably. Over extended times (e.g., greater than 2 hours), the surface area of the evaporating pool decreased sharply as the liquid evaporated. Under constant flux conditions, this behavior resulted in an evaporation rate that changed with time. More specifically, the mass loss curves showed a quadratic nature because mass loss was directly proportional to pool radius.

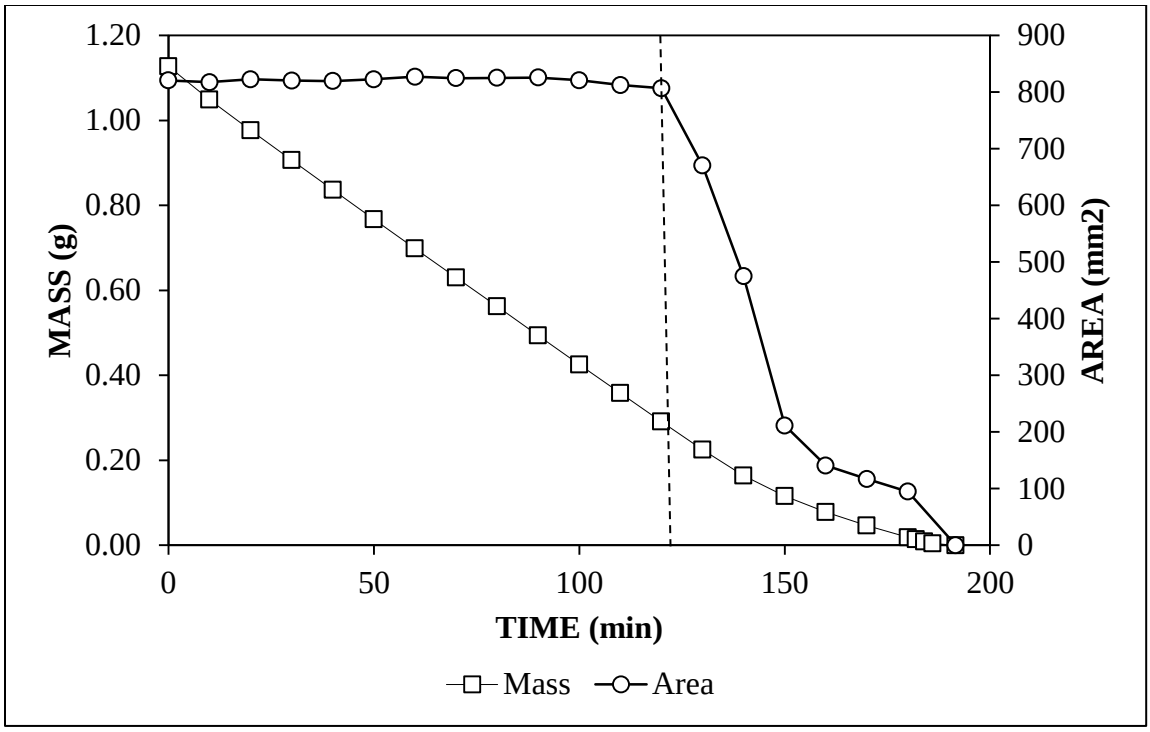


Figure 2.6: Mass Loss and Area Loss of Nitromethane.

Figure 2.7: Images of Mass Loss of Nitromethane. Note that both the surface area and radius of the liquid remain constant and then decreases with time after 120 minutes.

A direct comparison to the Deegan theory using Equation 1 was then performed. For example, the evaporation rate of a 1 mL pool of nitromethane after 10 minutes was estimated at 28 ºC and using an estimated diffusion coefficient for nitromethane of 0.112 cm^2/s. Equation 1 resulted in an evaporation rate of 0.0954 mg/sec for a pool of radius 1.82 centimeters (the radius of the pool after 10 minutes). For nitroalkanes other than nitromethane, the surface tension of the liquid was insufficient to form a distinct pool in

the sniffer tin and, therefore, the area could not be reliably measured. Regardless, it was also observed that the less volatile nitroaromatic liquids formed pools that did not change area due to their exceedingly slow evaporation rates (despite allowing them to evaporate for over 4 hours). As a result, mass loss experiments were conducted for the nitroalkanes where the liquid was confined to a Pyrex watch glass. The results of these experiments are shown in Table 2.3.

Table 2.3: Predicted versus Actual Evaporation Rates of Nitroalkanes. Summary of 1 mL quantities of unconfined nitroalkanes at 10 minutes.

Compound	Boiling Point (K)	Measured Radius (cm)	Temperature (K)	Predicted Evaporation Rate (mg/sec)	Measured Evaporation Rate (mg/sec)
Nitromethane	374	1.82	301	0.095	0.20
Nitroethane	387	2.02	298	0.070	0.11
Nitropropane	405	2.09	300	0.037	0.10

Although there is qualitative agreement between the Deegan theory and these results, the measured evaporation rates were approximately twice that of the calculated evaporation rates at the same time points. Potential sources of error in this determination include unintended convection currents within the balance chamber, deviations from the spherical cap geometry assumed by Deegan, and/or deviations of the vapor from the ideal gas law.

2.4. Conclusions

In this study it was determined that the evaporation rates of certain unconfined, nitrated liquids was dependent upon several factors including boiling point and sample amount (i.e., surface area). For example, the mass loss of nitromethane ranged from 0.018 mg/sec (for 10 µL) to 0.14 mg/sec (for 10 mL). Furthermore, the evaporation rate of the lighter nitromethane (61.04 g/mol) was approximately three times faster than the heavier nitropropane (89.09 g/mol). In all cases the mass loss curves fit a linear function over short times, an expected result given that evaporation rates are dependent upon "pinned" surface area. The evaporation rates for the liquids studied were found to decrease exponentially with boiling point. Although the qualitative behavior of the liquids agreed with Deegan's observations, the quantitative predictions of the theoretical model underestimated the evaporation of unconfined nitrated liquids.

There are several implications of these findings for canine training and testing. The first is that the surface area of an evaporating pool of liquid is an important variable as it directly influences the amount of odor that is generated. This implies that larger amounts of unconfined explosive will generate a larger amount of odor. However, the odor availability of a pure liquid explosive will decrease exponentially with increasing boiling point. Pure liquids will also produce a constant level of vapor as they evaporate until they "de-pin", whereupon the vapor production drops off in a quadratic fashion. Quantitative prediction of evaporation rates has proven difficult, hence an empirical approach is recommended for nitrated liquids.

CHAPTER 3. FLUX OF EXPLOSIVE VAPORS FROM PERFORATED CONTAINERS DESIGNED FOR CANINE TRAINING AND TESTING

3.1. Introduction

Because the degree of confinement can greatly affect the amount of explosive vapor that is released, understanding the movement of explosive vapors from partially sealed containers is a primary concern [24]. In the case of explosives-detecting canines, a standardized container with a defined headspace and degree of confinement has been developed [25]. This apparatus consists of a two ounce "sniffer tin" with a perforated lid used to hold small samples of explosive material (see Figure 3.1). When used for canine testing and training, the sniffer tin is placed inside a quart sized can to avoid disturbance by the canines upon detection. Finally, the quart can is placed inside a one gallon paint can to provide a defined headspace in which explosive odors collect for a minimum of 30 minutes prior to canine searching (see Figure 3.2).

a.) b.) c.)

Figure 3.1: a.) 2 oz. “sniffer tin” employed by the Bureau of Alcohol, Tobacco, Firearms, and Explosives for canine testing and training b.) sniffer tin top with asterisk patterned perforations c.) sniffer tin top with one, 1/8 in perforation.

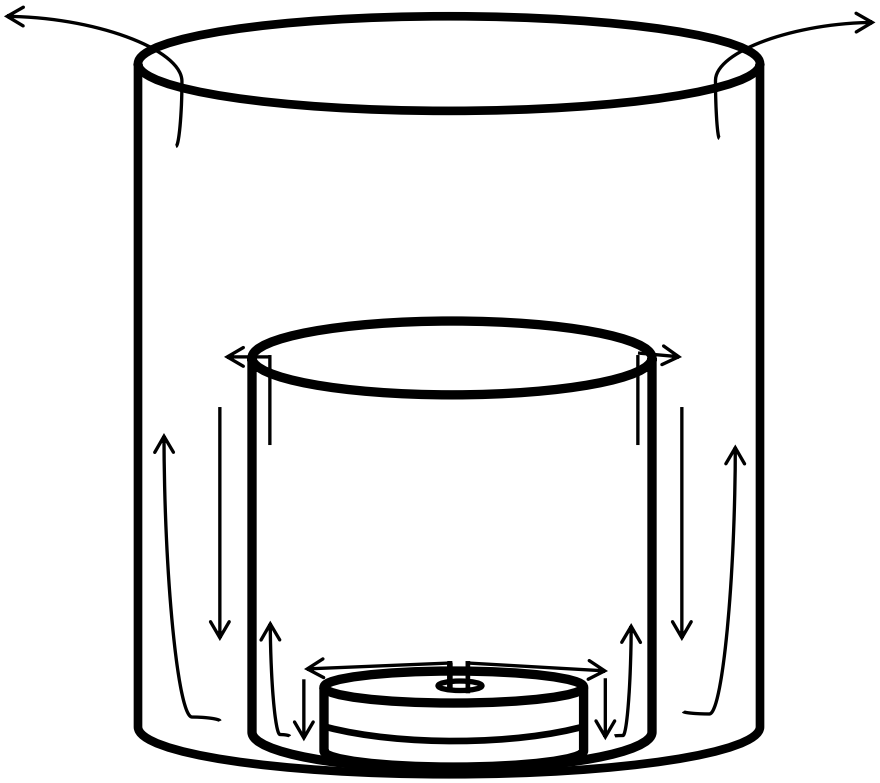


Figure 3.2: Standard Can Set-Up. Utilized in canine testing and training consisting of a 2 oz. sniffer tin inside a quart-sized paint can inside a gallon sized can. Arrows indicate vapor movement

Ultimately, the movement of explosive vapors within and out of such a container is dictated by mass transport. In turn, mass transport in gaseous media is governed by two main processes: diffusion, which is the result of random motion of molecules following concentration gradients, and convection, the bulk motion of a fluid or gas. The phenomenon of diffusion is the focus of this work and has been well described by Fick, who established several equations describing this phenomenon.

In particular, Fick's First Law of Diffusion defines flux as the amount of material diffusing through an orifice of a given area per unit time. In this model, the flow of material is assumed to be unidirectional and perpendicular to the orifice. When expressed as a differential equation, flux (J) is inversely proportional to the area (A) and directly proportional to the flow rate of material ($\frac{dn}{dt}$). This equation can also be written in terms of the diffusion coefficient (D) of the substance and its concentration gradient ($\frac{dc}{dx}$) across the orifice:

$$\boldsymbol{J = \frac{1}{A}\left(\frac{dn}{dt}\right) = -D\left(\frac{dc}{dx}\right)} \text{ (Equation 3.1)}$$

If these equations are rearranged and solved for flow rate, one can see that flow rate will be directly proportional to area, diffusion coefficient, and concentration gradient:

$$\boldsymbol{\left(\frac{dn}{dt}\right) = -AD\left(\frac{dc}{dx}\right)} \text{ (Equation 3.2)}$$

Furthermore, if a pure volatile substance is placed in a perforated container, a near-equilibrium should be established between the substance and its vapor. This, however, is only assumed if the rate of evaporation of the substance is significantly higher than the flow of material out of the container. In this case, the source of material may be considered infinite and the substance's headspace concentration is presumed to be fixed at $\frac{P^0}{RT}$, where P^0 is vapor pressure, R is the ideal gas constant, and T is temperature. This defines the case of unimolar diffusion, which is governed by the following integrated form of Fick's First Law:

$$\frac{dn}{dt} = -AD\frac{c_{in}}{\Delta x}\ln\frac{1-x_{out}}{1-x_{in}}$$ (Equation 3.3)

Where c_{in} is the concentration of the vapor inside the container, Δx is the length of the orifice (in this case the thickness of the sniffer tin lid). The natural log term represents the portioning of the vapor on either side of the hole, with x_{out} being the mole fraction of the vapor outside the container and x_{in} being the mole fraction of the vapor inside the container [14].

An even simpler approximation can be made by expressing the measurable quantity $\frac{dn}{dt}$ (in moles per second) as follows:

$$\frac{dn}{dt} = -AD\frac{dc}{dx} \cong -AD\frac{\Delta c}{\Delta x} = -AD\frac{c_{out}-c_{in}}{\Delta x}$$ (Equation 3.4)

where c_{out} is the concentration of the vapor outside the container, c_{in} is the vapor concentration inside the container, and Δx is the length of the hole (*i.e.*, the thickness of the lid). With the presumption that the vapor is diffusing from the container into a significantly larger volume, c_{out} may be assumed to be zero thus simplifying Equation 3.4 to the following:

$$\frac{dn}{dt} \cong -AD\frac{c_{in}}{\Delta x}$$ (Equation 3.5)

Ultimately, these models can be utilized to calculate the flow rate of material into the surroundings provided that the area and length of the hole in the container are known along with the diffusion coefficient and vapor pressure of the compound of interest. This is of keen interest when explosive vapors are confined in a perforated container and some portion of the explosive vapor escapes into the surroundings. The particular focus of this study was to evaluate whether Fick's First Law of Diffusion is a reliable means to calculate the amount of material that would be released from a small orifice as a function of time. If this is the case, the flux into the surroundings should be inversely proportional to the cross-sectional area of the orifice and directly proportional to the diffusion coefficient of the material (which is in turn dependent upon the molecular weight of the substance). In contrast, the flow rate of explosive material into the surroundings should be directly proportional to the cross-sectional area of the orifice as well as the diffusion coefficient.

3.2. Materials and Methods

Six nitrated liquids were used in this study: nitromethane (Sigma Aldrich, St. Louis, MO), nitroethane (Sigma Aldrich, St. Louis, MO), 1-nitropropane (Sigma Aldrich, St. Louis, MO), nitrobenzene (Fisher Scientific, Fair Lawn, NJ), 2-nitrotoluene (Alfa Aesar, Ward Hill, MA), and 3-nitrotoluene (Sigma Aldrich, St. Louis, MO). Sample containers were based upon those employed by the National Odor Recognition Test (NORT) for canine testing [16]. These containers are 2 ounce "sniffer tins" (Specialty Bottle, Seattle, WA) with perforated lids (see Figure 1). Lid perforations were made with a press purchased from Missile Engineering of Des Moines, Iowa.

Mass loss measurements of sniffer tins with holes of varying nominal diameters were completed to demonstrate the relationship to Fick's First Law of Diffusion. Each perforation was measured with calipers then averaged to obtain the actual diameter and area. All mass loss measurements were completed using 1mL volumes of explosive liquid. Mass loss measurements were obtained using an accuSeries accu-124 (Denver Instruments, Denver, CO) digital analytical balance. The accuSeries balance was connected through a USB connection to a Dell computer running Pinnacle USB software. The mass of the sniffer tin was recorded in two second intervals into a Microsoft Excel 2007 workbook. From this data, a plot of moles lost per unit time was created. The slope of this line was the flow rate ($\frac{dn}{dt}$). Predicted flow rates were determined at room temperature using Equation 3.5.

Diffusion coefficients were calculated using the Environmental Protection Agency (EPA) diffusion coefficient calculator available from their website (www.epa.gov) (see Table 3.1). The diffusion coefficient calculator uses the Fuller, Schettler, Giddings (FSG) method developed in 1966 to predict binary gas-phase diffusion [20].

Additional studies were conducted to determine the evaporative behavior of explosive mixtures as compared to pure explosives. The explosive mixture consisted of varying amounts of nitromethane and nitroethane prepared in solutions starting at 100% nitromethane and adding nitroethane in 25% increments until 100% nitroethane was reached. From here 1 mL of each solution was placed in a sniffer tin and its mass loss measured over time.

3.3. Results and Discussion

This study employed the use of six compounds with known explosive applications. All compounds are liquids at room temperature and have well known physical and chemical properties (see Table 3.1)

Table 3.1: Physical and Chemical Properties of Liquids Used in Flux Studies.

Compound	Molecular Weight (g/mol)	Boiling Point (K)	Vapor Pressure @ 298 K (Pa)[23]	Diffusion Coefficient @ 298 K (cm^2/sec)[b]	Application(s)
Nitromethane	61.0400	374	4790	0.122	Binary high explosives
Nitroethane	75.0666	387	2790	0.103	Binary high explosives
Nitropropane	89.0932	405	1360	0.0906	Binary high explosives
Nitrobenzene	123.1094	484	30.00	0.0767	Binary high explosives
2-nitrotoluene	137.1360	495	12.70[26][c]	0.0709	Taggant
3-nitrotoluene	137.1360	504	8.550[c]	0.0708	Taggant

[b] Estimated using EPA Online tools for site Assessment Calculation (www. epa.gov)
[c] Determined at 293.15 K.

The theoretical model discussed above states that the flow rate into the surroundings is linearly dependent upon the cross-sectional area of the orifice that is present in a container. Furthermore, the flux into the surroundings should also be linearly dependent on the diffusion coefficient of the substance, which in turn is dependent upon molecular weight. In this study, these predictions have been tested using gravimetric techniques, where the flow rates of various nitrated liquids were measured in sniffer tins as a function of hole diameter, number of holes, and temperature.

3.3.1. Flow of Vapor Through a Single Hole

The first set of experiments utilized 1 mL volumes of each nitroalkane and varied the diameter of a single perforation in the sniffer tin lid. When the resultant flow rates are expressed as a function of hole area, a clear curvature is evident in the data (see Figure 3.3).

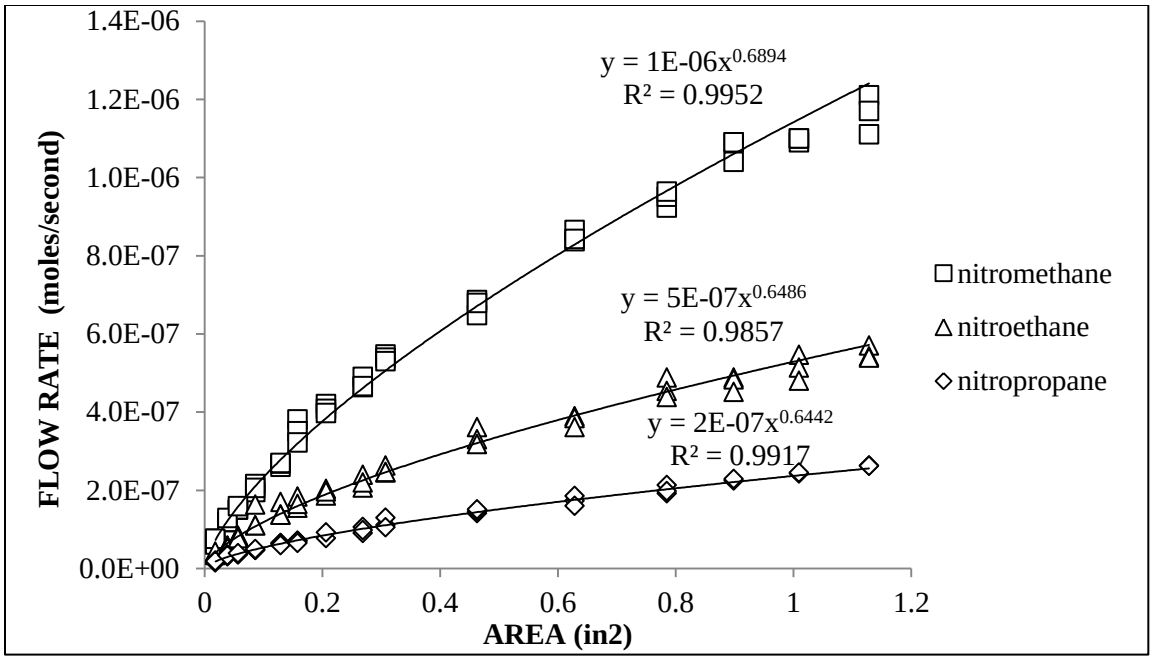


Figure 3.3**:** Mass Loss of Nitroalkanes as a Function of Hole Area

The curvature in the data was seen even at hole sizes down to 1/8-inch diameter; as the hole area increases beyond this point, the flow of material begins to rapidly level off. The data, in fact, fit well to a power function with fractional exponents of 0.64, 0.65 and 0.69 for the three nitroalkanes. An interesting and puzzling result upon gathering this data was that when the data was plotted as the measured flow rate versus nominal hole diameter (not shown), a directly proportional relationship is seen with excellent linearity ($R^2 > 0.99$). Using Fick's First Law of Diffusion, the predicted flow rate of nitromethane was calculated at room temperature and compared to the measured values (see Figure 3.4). In Figure 3.4, a directly linear relationship is seen between flow rate and hole area, however a clear curvature is again seen in the measured data.

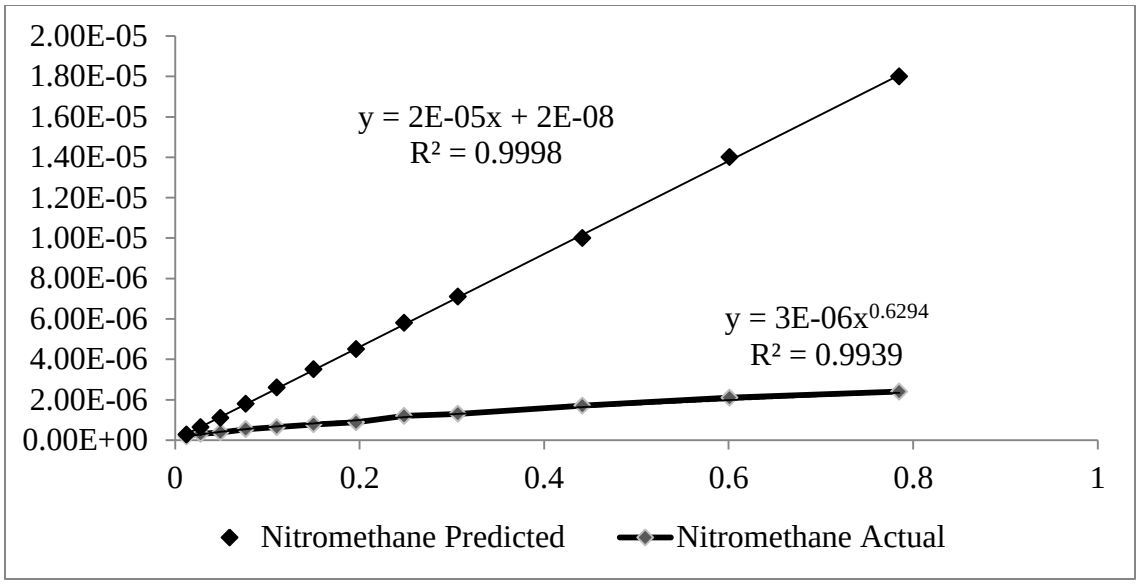


Figure 3.4: Measured versus Predicted Mass Loss of Nitromethane.

Therefore, in order to more fully understand the flow dynamics of the system, the flow from several sniffer tins was visualized by placing a small amount of water and dry ice into the tin and video recording the flow of white fog as it left the tin. Under highly constrained conditions, the fog emerged as a jet from the sniffer tin, approximately at a 90 degree angle with the sniffer tin lid. As hole size was increased, however, the fog clearly emerged along the circumference of the hole, radiated outward along the top of the tin surface and cascaded down the sides of the tin.

Taken together, these observations imply that flow rate is not directly proportional to cross-sectional area in this system. The most likely reason for this is that assuming a unidirectional flow perpendicular to the orifice is invalid with large hole sizes. Instead, a flow pattern that is limited by the circumference of the hole, not its area, is dominant. The geometry of the system also dictates that if flow rate is linearly dependent upon

diameter, then it should be directly proportional to the square root of the area. It is suggested that the fits of the data in Figure 3.3 to functions whose exponents are close to 0.5 is therefore not coincidental.

3.3.2. Flow of Vapor Through Multiple Holes

While understanding the flux of explosive vapors from containers with one perforation is of interest, in realistic circumstances containers will likely have more than one hole through which vapors will escape; therefore, flux from containers with more than one hole was explored.

For example, the effect of a collection of smaller holes producing flow rates equivalent to larger holes was studied by measuring the mass loss of nitroalkanes in sniffer tins with an increasing number of 1/8 nominal diameter holes. The results, shown in Figure 3.5, showed a notable and linear increase in flow rate as a function of hole number.

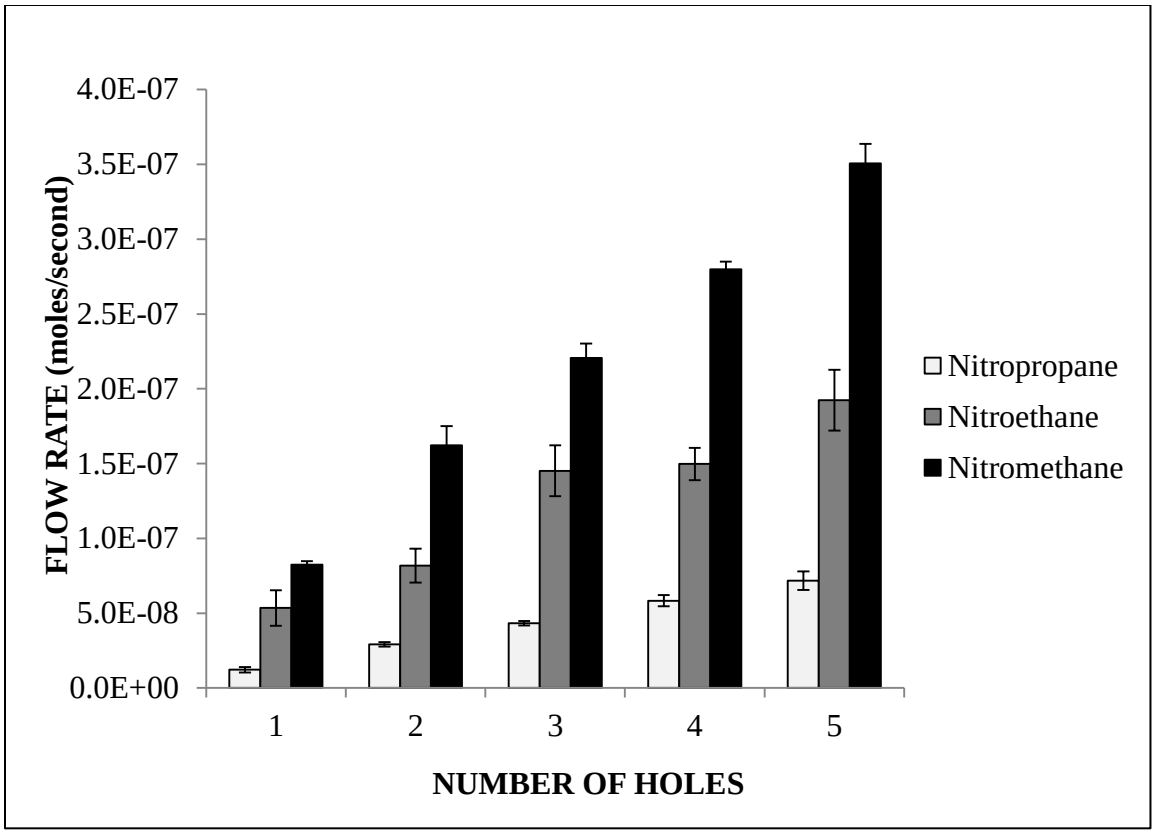


Figure 3.5: Mass Loss of Nitroalkanes as a Function of Hole Number.

It was also noted that on a per unit area basis, a collection of 1/8-inch holes generates higher flow rates than the corresponding holes of a larger diameter. This indicates that the loss of flow due to diffusion that is not unidirectional is an important factor for any hole larger than 1/8-inch in diameter.

A standardized press that creates numerous holes of 1 mm^2 area in an asterisk pattern was acquired (see Figure 1a). Using 1 mL quantities, the flow rates of the three nitroalkanes were determined and compared to the flow rate from tins with a single hole. In this case, the flow of nitromethane from a standard sniffer tin was equivalent to the flow from a tin with one, relatively large hole (~0.2 in^2 or 0.5 inch diameter). This experiment was repeated with the remaining nitroalkanes with similar results.

To verify the assumption that the flow rate from a sniffer tin would be independent of sample amount, additional measurements were completed in standard sniffer tins with varying amounts of liquid (1 μL–10 mL). For example, the calculated flux from a sniffer tin showed no significant change when the sample amount was increased from 1 mL (4.46×10^{-6} mol/in^{2}s) to 3 mL (4.37×10^{-6} mol/in^{2}s). In general, it was assumed that the amount of explosive material present in a perforated sniffer tin would not affect the rate of flow so as long as the amount of material present was sufficient enough to saturate the container. This assumption was confirmed by placing increasing volumes of nitromethane into a standard sniffer tin and measuring the flow rate that resulted (see Figure 3.6).

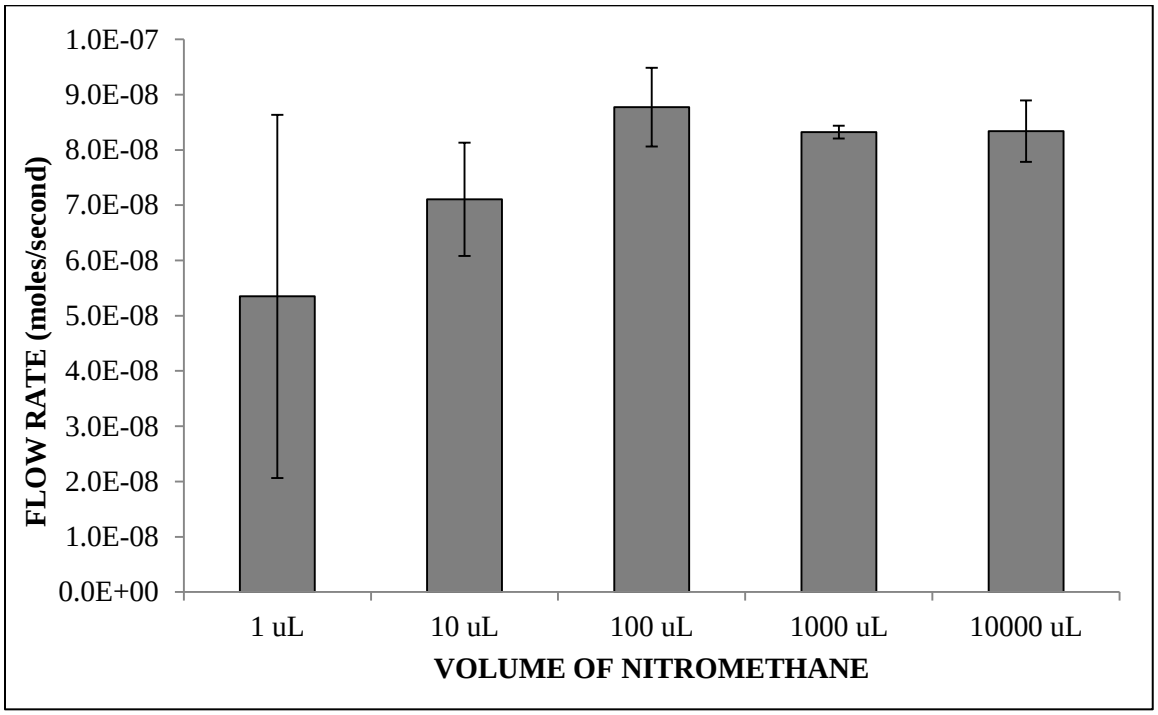


Figure 3.6: Effect of Sample Volume on Flow Rate.

3.3.3. Temperature Effects

The effect of temperature was also of interest as vapor pressure and diffusion coefficient increase with increasing temperature, leading to increased odor availability. Through use of a moisture balance provided by Dow Agrosciences (Indianapolis, IN), mass loss measurements were taken at 40 ºC (104 ºF) and compared to those obtained at room temperature (25 ºC). As expected, the flux of vapor from a standard asterisk patterned sniffer tin was highly temperature dependent, resulting in a nearly three-fold increase in flow rate as compared to those obtained at 25 ºC (see Figure 3.7). This is likely the result of two factors. First, there will be an increase in the diffusion coefficient with increasing temperature, leading to increased flow through an orifice. Second, as stated above, higher temperatures result in higher vapor pressures and, hence, a higher concentration of vapor inside the sniffer tin that can drive mass transport.

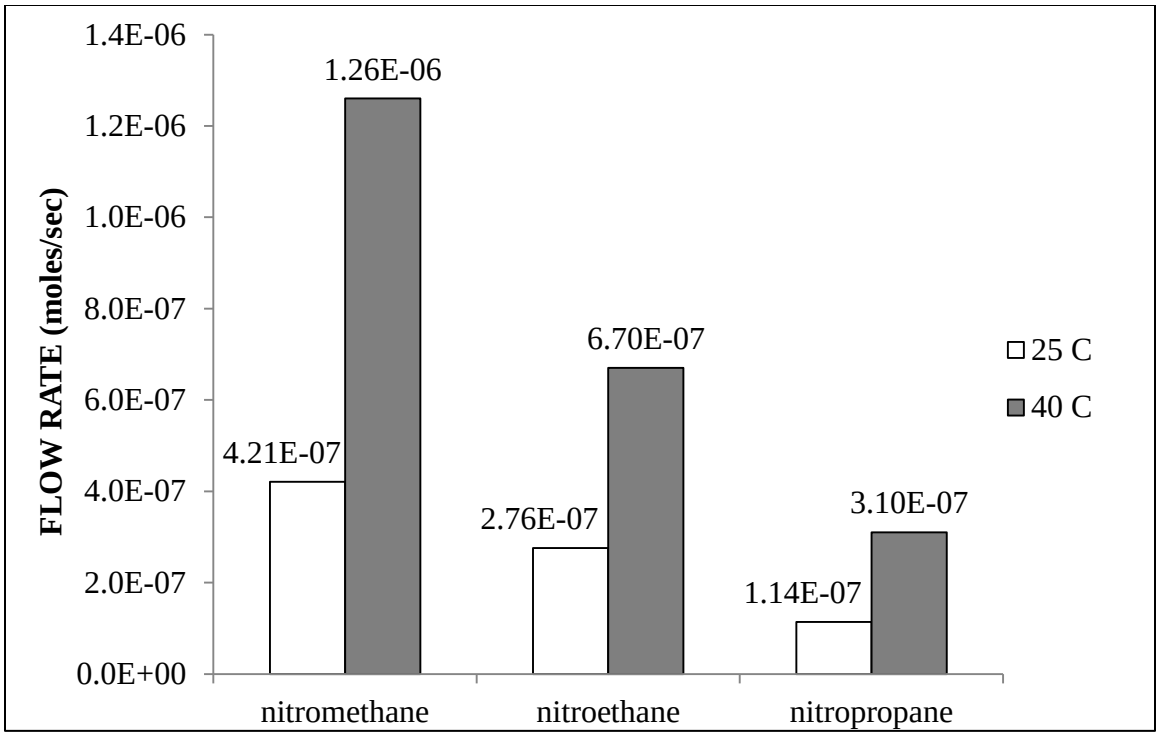


Figure 3.7: Effect of Temperature on Flow Rate.

3.3.4. Flux of Vapors from Explosive Mixtures

Finally, the flow rate of nitromethane was measured upon mixing with ammonium nitrate to demonstrate the evaporative behavior of the explosive Kinestick, a binary high explosive. In this case, the flow rate of the nitromethane/ammonium nitrate mixture (2.1×10^{7} mol/sec) was approximately 10 times lower than that of pure nitromethane (2.1×10^{-6} mol/sec). The flow rate of mixtures of liquids has also been explored by varying the ratio of nitromethane and nitroethane in the mixture. With these studies it was determined that explosive mixtures exhibited slower flow rates as opposed to the pure compounds (see Table 3.2). These differences can be attributed to the change in vapor pressure and diffusion coefficient as the mixture becomes less pure.

Table 3.2: Measured Evaporation Rates of Nitromethane/Nitroethane Mixtures.

Mixture	Evaporation Rate (moles/second)
100% NM	2.09E-06
75% NM	8.44E-07
50% NM	8.22E-07
25% NM	7.69E-07
100% NE	1.04E-06

3.4. Conclusion

The behavior of pure explosive liquids when they are placed in containers with small orifices has been studied and compared to Fick's Laws of Diffusion. It has been determined that the flow rate of vapor through a small hole is dependent upon several factors including the geometry of the hole (*i.e.*, area, thickness), the concentration gradient across the hole, the diffusion coefficient and vapor pressure of the liquid, as well as the temperature.

Although the effects of diffusion coefficient and concentration gradient were proven, overall it was shown that Fick's Laws can only apply to containers whose perforations are less than 1/8-inch in diameter. As the cross sectional area increases, the proportionality between flow rate and hole area decreases. Instead, flow rates were directly proportional to hole diameter (circumference).

In general, holes of smaller diameter generated the greatest flux (flow rate per unit area), while holes with larger diameters generated the largest flow rate into surroundings. Based on this finding, it has been shown that a sample container geometry consisting of multiple holes of a small diameter yields a higher flow rate per unit area than does a single hole of larger diameter. This will explain why a conventional sniffer tin (as used by NORT) can generate a significant flow rate despite having smaller holes. It has also been shown that a perforated container will produce the same flow of vapor regardless of the amount of pure substance present inside of it.

Overall, this study has shown that if sufficient material is present and enough time is allowed for the vapor to flow into the secondary containers, then the actual amount of material and its confinement has no impact on detection success. This theory will be tested using certified explosives-detecting canines and the results shared later in Chapter 5.

CHAPTER 4. EXPLORATION OF 2, 4-DINITROTOLUENE AND NITRATE AS EXPLOSIVE ODOR COMPOUNDS

4.1. Introduction

Although canines are regarded as one of the most effective real-time detectors for explosive devices, controversy still remains regarding several key concepts. One in particular involves the characterization of the chemical vapors being emitted by explosive formulations. While studies have been conducted to identify these "odor compounds" most are inconclusive or have identified species unrelated to the explosive itself. Difficulties in isolation and identification of odor compounds arise from the fact that odors generated from explosives are often complex, therefore their headspace will consist of multiple chemical compounds. These compounds may be degradation products that originate from the explosive itself, plasticizers in the case of plastic bond explosives, energetic "taggants" that are added to the explosive to increase detection success, or are more volatile "signature compounds" originating from additives, solvents, or explosive impurities.

While pure explosives exhibit low vapor pressures the odor compounds generated often have significant volatility. This introduces the possibility of odor compounds as a source of canine alert. An example of this complex odor generation is trinitrotoluene (TNT) whose vapor consists of the extremely volatile compounds dinitrobenzene (DNB) and dinitrotoluene (DNT). Previous studies exploring the possibility of DNT as a potential cause of canine alerts to TNT have been conducted. Because TNT is the primary explosive comprising land mines, studies concerning their detection have been conducted using gas chromatography/mass spectrometry (GC/MS) where DNT isomers were identified in post-blast residues of TNT landmines [27]. Additional studies have been reported concerning the detection of 2, 4-DNT vapor using surface enhanced Raman spectroscopy and artificial nose technology [13, 28].

Also of interest is the possibility that nitrated explosives naturally produce nitric acid which in turn may produce nitrated gases (i.e., nitrous oxide, nitrogen dioxide, or dinitrogen oxide). To date, nitric acid has not been explored as a possible cause of canine alerts. The purpose of this chapter is to explore the possibility of nitric acid as an "explosive related compound" (ERC), a concept that has not been previously explored, and to study the properties of the DNT isomer 2, 4-DNT and its function as an odor compound.

4.1.1. The Nitrate Hypothesis

The formation of nitric acid upon decomposition of nitrated explosives has been reported [29]. Furthermore, it has been proposed that it is these nitrated gases that may be the cause of canine alert to nitrated explosives. The formation of nitric acid by a nitrate ester, such as nitrocellulose, upon reaction with atmospheric water proceeds as follows:

$$\mathbf{R\text{-}O\text{-}NO_2 + H_2O \longrightarrow R\text{-}OH + HNO_3}$$

$$\mathbf{4HNO_3 \longrightarrow 2\,H_2O + 4NO_2 + O_2}$$

The formation of these de-nitrated species indicates the generation of nitric acid by the base explosive. This reaction is well known for the secondary high explosive nitrocellulose, were the addition of the stabilizer diphenylamine is required to act as a proton scavenger to inhibit the formation of nitric acid [30]. This effect is supported by the knowledge that non-volatile, unbound, nitrated explosives are readily detected by canines, indicating the parent explosive must be emitting some compound that facilitates this alert.

While this mechanism holds true for nitrate esters, this reaction would not be expected for all nitrated explosives. For example, the decomposition of the nitroaromatic explosives TNT and Composition C-4 would not likely result in the formation of nitric acid. Instead, the water reacting with the aromatic compound would simply result in the de-protonation of the ring and the formation of hydronium ion. The goal in this portion of the study is to eliminate nitric acid as a potential ERC of the explosives TNT and Composition C-4. Because the explosives of concern in this study have little to no

volatility at room temperature (*i.e.*, TNT, Composition C-4, and their deactivated analogs in the form of Non-Hazardous Explosives for Security Training and Testing (NESTT)), analysis by LC/MS is necessary. An LC/MS method for the detection of nitrate ions in black powders has been developed [31]. However, because this study is focused on TNT and Composition C-4, some method development is required. There are two implications of the lack of nitric acid formation by nitroaromatic explosives. First, due to the acidic nature of the solution after de-protonation, each explosive will have in a measurable pH of < 7.0. Second, the detection and quantitation of nitrate ions in aqueous extracts will be not be possible via LC/MS.

4.1.2. DNT Residues

While identification of potential odor compounds is important, the mechanisms behind their emission from the base explosive is also of concern, particualy the amount of vapor available for sampling. Most odor compounds are significantly more volatile than their parent explosives. However, when placed in a container, the amount of vapor emitted is not necessarily equivelant to the amount of vapor available for detection. This is due to the "sticky" nature of the explosive vapors themselves which results in some amount of vapor adhering to the surface of the container in which it is confined. A study concerning the movement and availability of explosive vapors was conducted and shared in Chapters 2 and 3. In these studies explosive vapors were shown to disperse out and fill the container in which they are confined from the bottom (see Figure 2.1). These studies

also included mass loss measurements where the flow rate of various nitrated liquids were determined, however, quantitation of the amount of explosive adhereing to the container surface was not explored.

According to the mechanism in section 4.1.1, 2, 4-DNT is a product of TNT degradation. Additionally, studies have introduced 2, 4-DNT as a trigger for detection of TNT landmines. Because 2, 4-DNT has been identified as a potential odor compound in several studies, it proves to be an ideal canditate for studying the adhesion of explosive vapors on container walls. To date, several methods for the detection of explosives, inlcuding 2, 4-DNT by GC/MS and solid-phase microextraction (SPME) have been reported [32, 33] This study, however, concerns detection of 2, 4-DNT in inorganic extracts, therefore Liquid Chromatography/Mass Spectrometry (LC/MS) may be most suitable. Also due to its physical similarities to 2, 4-DNT, the use of 2, 6-DNT as an internal standard will be explored therefore separation of these isomers will be nessary. Once a method for the detection and separation of 2, 4-DNT is developed, extraction and quantitation of its residues will be attempted.

4.2. Materials and Methods

Calibrants were prepared using Ammonium Nitrate (Fisher Scientific, Rochester, NY). Solutions were diluted with HPLC grade water purchased from Fisher Scientific (Rochester, NY). The column used was a Hypercarb (2.1 x 100 mm x 5µm) purchased from Thermo Scientific (Waltham, Massachusets) and set at 30 ºC. A 1 % Formic Acid in water mobile phase was set to flow at 400 µL/min with an injection volume of 5 µL. All NESTT materials, flake TNT, and Compositions C-4 were provided by the Bureau of Alcohol Tobacco Firearms and Explosives (ATF). Whatman and Gelman nylon filters (Fisher Scientific, Rochester, NY) were used to remove undissolved solids for solutions prior to injection.

4.2.1. 2, 4-DNT Detection

Calibrants were prepared in solutions using 99% 2, 4-DNT (Spectrum, Gardena, CA), 99% 2, 6-DNT (Sigma Aldrich, St. Louis, MO), and acetone (Fisher Scientific, Rochester, NY). A stock solution of internal standard was prepared at 65 ppm by placing 6.5 mg of 2, 6-DNT in 100 mL of acetone. This solution was diluted again with 100 mL of acetone resulting in a 650 ppb internal standard solution. A 1,000 ppm 2, 4-DNT stock solution was prepared by placing 5 mg of 2, 4 DNT in 5 mL of internal standard solution. From here calibrants were prepared by placing 1 mL of internal standard solution in a 10 mL volumetric flask with increasing amounts of 2, 4-DNT stock solution followedby dilution to volume with acetone.

Because this study focused on the detection of trace quantities of explosives, calibrants were prepared with low concentrations of 2, 4-DNT starting at 0.2 ppm by adding 2 µL of 2, 4-DNT to the solution described above and ending with the addition of 12 µL. This concentration window was decided upon after preliminary residue extractions showed the amount residues adhering to the container walls to fall within this window.

4.2.2. pH Measurement of Explosive Compounds

Prior to instrumental analysis, pH measurements were conducted on TNT, Composition C-4, and various NESTT materials to detemine acidity. All pH measurements were collected using a Fisher Scientific Acccumet glass AgCl pH electrode equipt with a 30" cable and BNC connector. Solutions were prepared by placing 2 grams of material in 20 mL of dionized water. After submerging the electrode, the solution was stirred for five minutes using a Fisherbrand teflon magnetic stir bar to allow the electrode to stablize. Once stablized, pH measurements were collected in five minute intervals for thirty minutes. Each compound was measured in triplicate with calibrations conducted between each trial.

4.2.3. Instrumental Analysis

4.2.3.a. Nitrate Detection

Because there are few studies concerned with nitrate detection via LC/MS some method development was required to detemine optimum detection parameters. In a study conducted at Indiana University Purdue University Indianapolis, successful detection of anions (including NO_3^-) in inorganic explosive powders was achieved [31]. This High Performance Liquid Chromatography (HPLC) method utilized a Porous Graphitic Carbon (PGC) column (2.1 x 100 mm) coupled with electrospray ionization mass spectrometry. This method was used to provide a basis for the detection of nitrate ions in aqueous extracts.

This study utilized a Thermo Scientific Accella HPLC equipped with a PDA, Pump, and Autosampler coupled with a Surveyor MSQ Plus detector. The mass spectrometer was set in Selected Ion Mode (SIM) for m/z 62.00 (mass of NO_3^-) with a cone voltage of 75 eV and ion source temperature of 550 ºC. The column temperature was set at 80 ºC and the flow rate at 400 µL/min using a 1% Formic Acid in water mobile phase solution and 1 µL injection volume.

To test the method, calibrants were prepared at various concentrations. A stock solution was prepared by dissolving ~10 g of ammonium nitrate in 100 mL of HPLC grade water resulting in a 1 mM nitrate solution. Calibrants were prepared by taking 2,4,6,8, and 10 mL volumes of the stock solution and diluting to 100 mL with HPLC grade water. Once an acceptable calibration was achieved, the least concentrated

calibrant was injected multiple times and the data observed for consistency. These results showed a loss of sensitivity after each injection, a likey result of the high column temperature (80 ºC) and highly concentrated mobile phase (1% Formic Acid in water).

In order to increase sensititivy the column temperature was decreased to 30 ºC, the injection volume increased to 5 µL, and the injection mode changed from partial loop to full loop. Next, a study was conducted to determine the best mobile phase composition. Using 1% formic acid, an isocratic study was performed starting at 100% Formic Acid solution and increasing the water content by 10% with each run. This study showed an decrease in both sensitivity and peak resolution as more water was added to the mobile phase compostition. An increase in retention time was also seen (see Figure 4.1) therefore the 100%, 1% Formic Acid solution was determined to provide the best results.

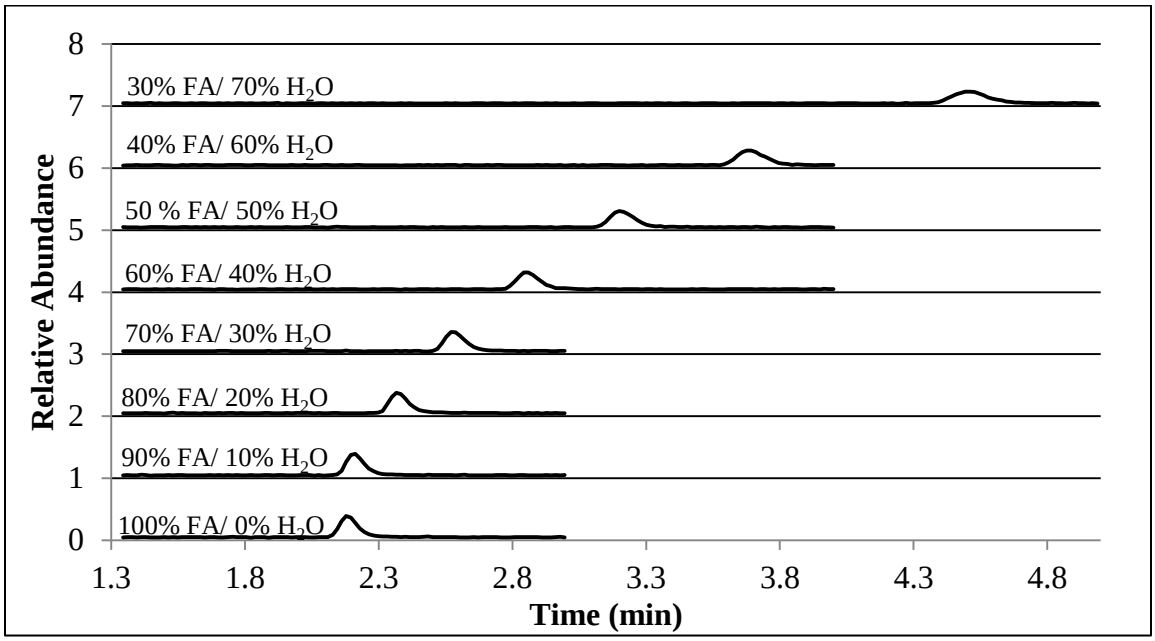


Figure 4.1: Mobile Phase Determination for Nitrate Detection.

4.2.3.b. 2,4-DNT Detection

Because the extracts were in the aqueous phase, analysis via LC/MS was attempted using the Thermo Scientific HPLC described above with a Hypersil Gold (50 x 2.1 mm x 1.9 µm) C_{18} column. Although successful detection of TNT, 2, 4-DNT, and 2, 6- DNT did occur, when placed in a single solution, separation of the 2, 6-DNT and 2, 4-DNT isomers proved problematic. Various mobile phase compositions were attempted using methanol and water, however, complete resolution did not occur and analysis by LC/MS was abandoned.

Detection and separation of the extracts from the interior surface of cans was attempted using a Thermo Trace GC Ultra equiped with DSQ II detector and DB-5MS (30 m x .25 mm x .25 µm) column. For optiminal sensitivity the mass spectrometer was set in selected ion-negative ionization chemical ionization (SIM-NICI) mode. The ion source temperature was set at 200 ºC and the inlet temperature was set at 40 ºC. The PTV was operated under splitless flow for 0.5 minutes with a split flow of 40 mL/min. The initial oven temperature was 40 ºC with a 20 ºC/min ramp to 220 ºC and held for 1 minute. This method used methane reagent gas flowing at 2 mL/min. Using this method, successful detection and separation of trace amounts of TNT and DNT isomers was possible (see Figure 4.2)

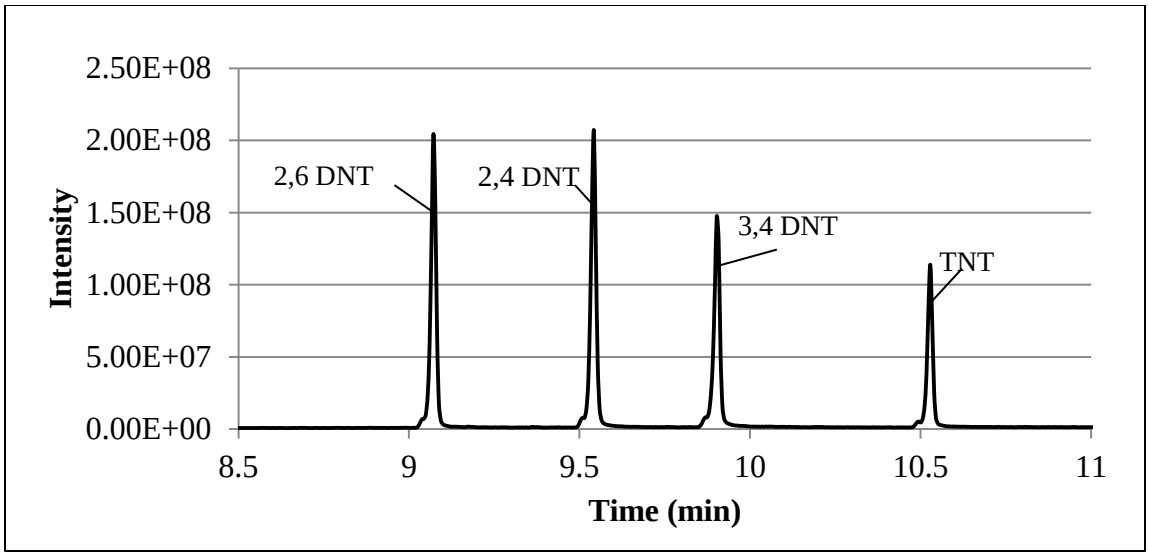


Figure 4.2: GC/MS Separation of TNT and DNT Isomers.

4.2.4 Extractions

4.2.4.a. Nitrates

Using TNT-NESTT, various extraction techniques were attempted using Chloroform and HPLC water as extraction solvents. The first method attempted extracted 100 mg of TNT-NESTT in 1 mL of Chloroform (Fisher Scientific, Rochester, NY), vortexing for 60 seconds, adding 2 mL of of HPLC water, vortexing again for 60 seconds, and finally collecting the aqueous layer and filtering before injection using a Whatman 0.2 µm Nylon filter with GMF. The same procedure was repeated using HPLC water instead of Chloroform and injecting the aqueous solution. The above procedure resulted in a small nitrate peak in the TNT-NESTT extract using both water and Chloroform solvents, however a small peak was also observed in the control (see Figure 4.3). Ultimately it was determined that the nitrate peaks that were observed were the result of carryover from the Whatman filters used before injection.

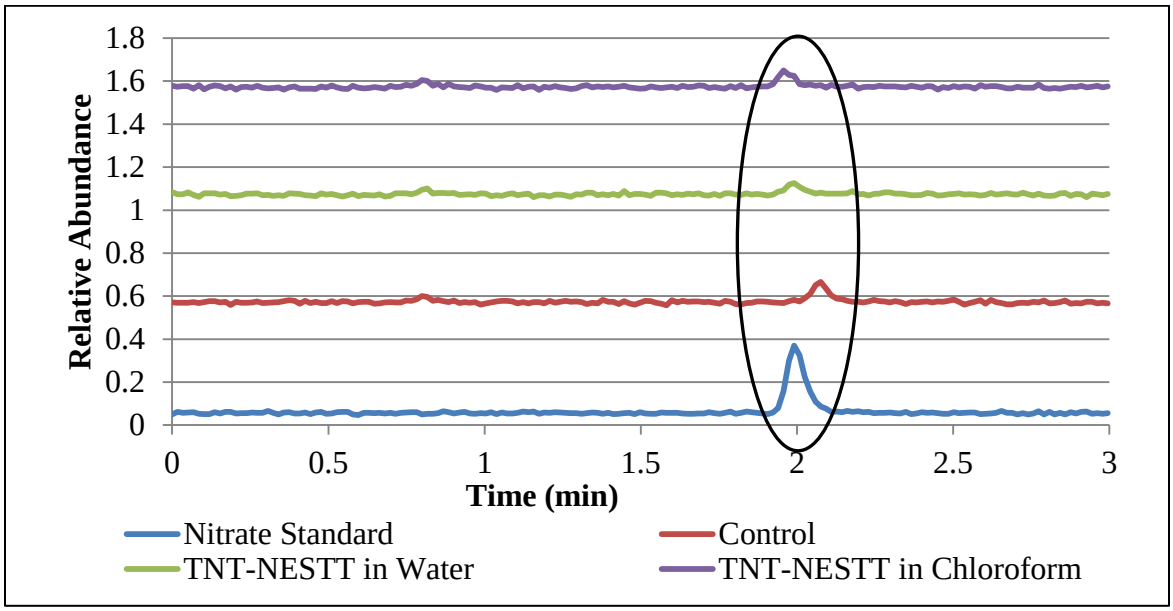


Figure 4.3: Nitrate Detection in TNT-NESTT.

Because nitrate ions were observed in the filters used in the extraction process a new extraction technique was necessary. A test was conducted to determine the volume of water necessary to rinse the nitrate ions from the filters and it was determined that 4 mL of HPLC water would clear the filters of any contaminants. The above procedure was repeated with the addition of running 4 mL of HPLC water through the filters prior to filtering the extracts. Unfortunately, these injections resulted in no nitrate detection in any of the extracts tested.

Additional extractions were attempted on the NESTT materials and on the TNT degredation product, 2, 4-DNT, with no successul nitrate detection. Previous extractions on these materials, particularly the nitrate NESTT showed HPLC water to be a more effective extraction solvent providing clear, detectable peaks using the above HPLC method. Still using water as an extraction solvent, a new procedure was attempted by dissolving 200 mg of material in 2 mL of HPLC water and ultrasonacating for 60 minutes

before filtering using 0.2 µm Gelman Nylon filter. While this extraction was ineffective for RDX-NESTT, TNT-NESTT, and 2, 4-DNT, an acceptable peak was visable for the nitrate standard and Nitrate-NESTT (see Figure 4.4). Because this procedure proved to be the most effective of the extraction techniques attempted it was used in the extraction of the explosives TNT and Composition C-4.

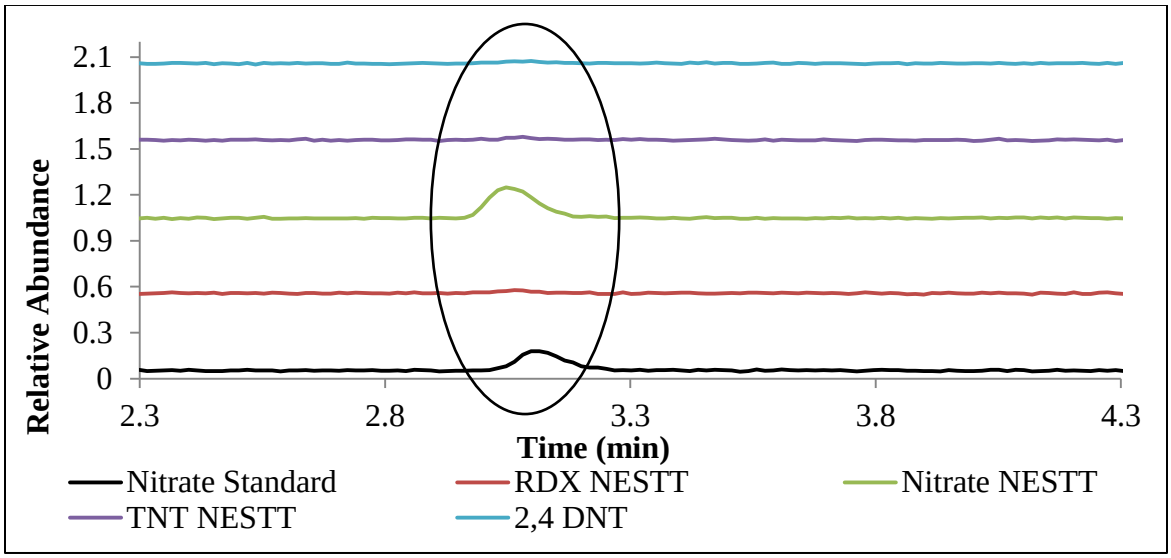


Figure 4.4: Aqueous Extraction of NESTT Materials and 2, 4-DNT.

4.2.4.b. 2,4-DNT Extraction

The containers were set up according to the National Odor Recognition Test (NORT) described in Chapter 3 (see Figure 3.2); the unconfined sniffer tin (Specialty Bottle, Seattle, WA) was placed within an unconfined quart can, which was placed inside a sealed gallon sized can (W.W. Grainger Inc., Indianapolis, IN).

After placing 100 mg of 2, 4 DNT in an unconfined sniffer tin, the gallon can was sealed and allowed to equilibrate for 24-48 hours. Extracts were then collected from the quart and gallon sized cans by placing 10 mL of internal standard solution in each container. The containers were then resealed and swirled for 5 min to collect residue. This solution was then aliquoted into a GC/MS vial and analyzed.

4.3. Results and Discussion

4.3.1. Nitrate Detection

pH measurements were taken for TNT, 2, 4-DNT, and Composition C-4. Various NESTT materials were studied in addition: RDX, TNT, and PETN-NESTT. Two additional NESTT matierals were tested to serve as standards; Nitrate-NESTT, known to contain nitrates, and Silca Distractor, a silica coated sand particle with no nitrate present. The results of these measurements are shown in Table 4.1 where pH was recorded every five minutes for three, thirty minutes trials. As expected, each compound tested was at least slightly acidic, supporting the theory of de-protonation accuring upon reaction with atmospheric water.

Table 4.1: Measured pH of Explosives and Related Compounds.

Compound	Average pH		
PETN-NESTT	6.43	+/-	0.023
RDX-NESTT	6.34	+/-	0.082
Silica Distractor	6.27	+/-	0.089
TNT-NESTT	6.03	+/-	0.053
TNT	5.93	+/-	0.019
Nitrate-NESTT	5.90	+/-	0.073
C-4	4.79	+/-	0.050

Several extractions were performed on the NESTT materials and 2, 4-DNT to detemine the most effective nitrate extaction procedure. While virtually no nitrates were detected in these materials, an effective extraction technique was developed as evident by the Nitrate-NESTT extractions. Because the NESTT materials contain significantly small quantities of actual explosive (~8%), the above extraction procedure was performed on flake TNT and Composition C-4 to detemine the presence of nitrates in actual explosives

(see Figure 4.5). In these extractions, no nitrate was observed in either TNT or Composition C-4. The extractions shown in Figure 4.5 were performed after a successful instrument calibration where the concentration of the lowest calibrant was 5 ppm. Theoretically, if nitrates were indeed present in the actual explosives, one would expect to observe them in the extracts. However, because the detection limit of nitrates by HPLC is unknown, the possibility of a nitrate presence below the detection limit exists.

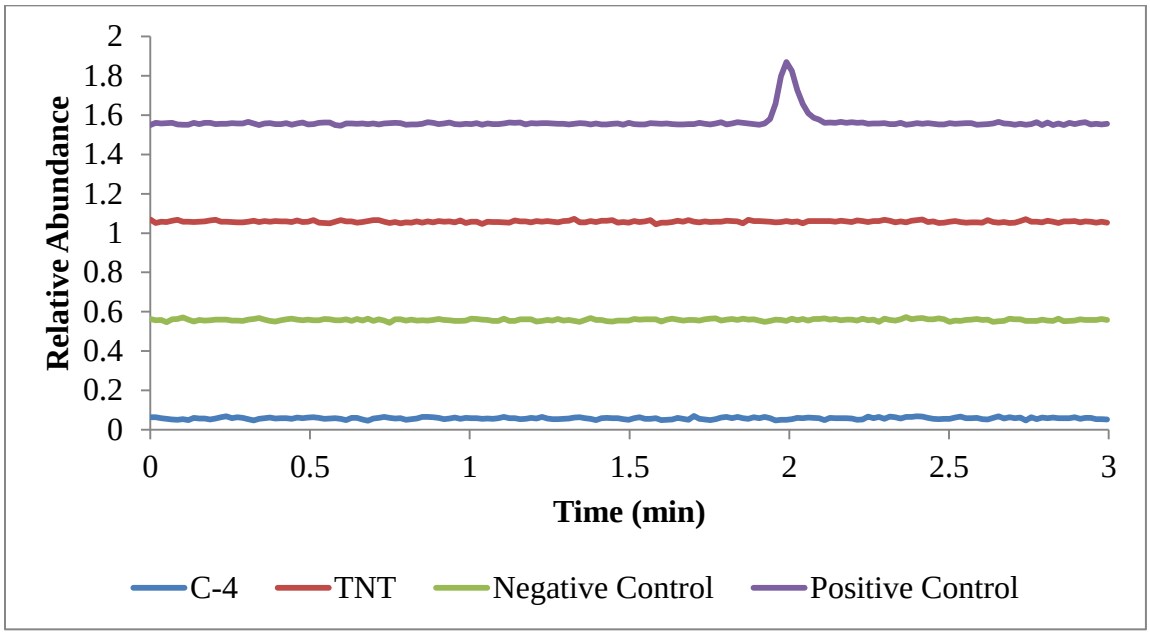
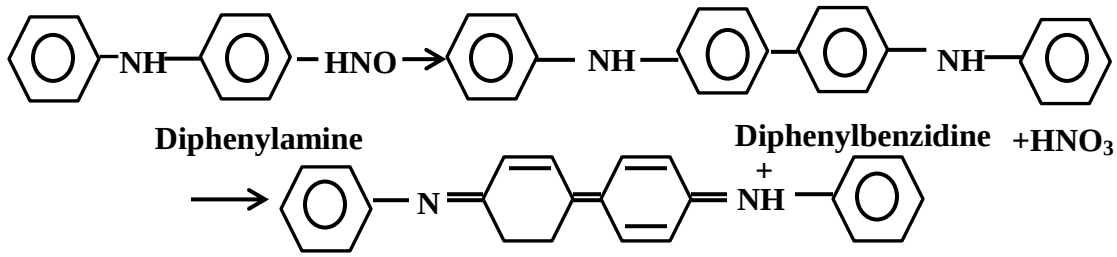


Figure 4.5: Nitrate Detection in TNT and Composition C-4.

Because instrumental analysis was ineffective for nitrate detection in explosives, a simple color test was performed to confirm the lack of nitrate ions in TNT and Composition C-4. The color test reagent was prepared by adding 10 mL of concentrated

sulfuric acid (Fisher Scientific, Rochester, NY) to 2 mL of deionized water followed by the addition of 50 mg of diphenylamine (DPA) (Fisher Scientific, Rochester, NY) while stirring [34]. The reagent works according to the following reaction:



Blue Quinoid Immonium Ion

Solutions were prepared by adding 200 mg of explosive to 20 mL of deionized water and stirring for 30 min. TNT, Composition C-4, and TNT-NESTT were tested along with Nitrate-NESTT to serve as a positive control. After stirring, 0.5 mL of DPA reagent was added to 1 drop of test solution in a spot plate and the reaction observed (see Figure 4.6). According to the DPA color test, TNT, Composition C-4, and TNT-NESTT did not contain nitrate ions. While these explosives produced slightly acidic solutions, subsequent results were in aggreement with the nitric acid hypothesis proposed.

Figure 4.6: Results of DPA Color Test for Nitrates. From the left: TNT, C-4, TNT-NESTT, Nitrate-NESST, and H_2O.

4.3.2. 2,4-DNT Residues

A method for the detection and separation of DNT and its isomers via GC/MS was developed. Using this method, quatitation of the amount of 2, 4-DNT residue adhering to a container's walls was attempted. Solutions were ran at approximately 0.2 ppm-1.2 ppm to create a calibration curve. Each solution and extract was ran in triplicate and the response plotted as a function of concentration (mg/mL). The linear regression resulted the equation, $y = 12170x - 1.374$ with and R^2 value of .975 (see Figure 4.7). Using the linear regression equation, the amount of 2, 4-DNT residue on the inside of a quart and gallon can was calculated. The average mass of 2, 4-DNT adhered to the sides of a quart sized can was calculated to be 6.6 µg while the amount of residue on the sides of a gallon sized can was 2.5 µg.

Using the Ideal Gas Law the amount of material expected in the headspace was caluclated for both the gallon and quart sized cans. These results differed significanty from those obtained from containers with expected values being 5.19 µg and 1.29 µg for a gallon and quart sized can respectively. These results indicate that while only minute quantities of material are required to fully saturate containers, the abundance of 2, 4-DNT in the solid phase compared to the gas phase results in a significant amount of material being lost to surface adhesion. This loss can greatly affect the amount of material available for canine sampling when training canines to detect trace amounts of explosive.

The amount of material recovered from the quart can was significantly greater than that recovered from the gallon sized can. This was unexpected after conducting the dry ice experiment which indicated that vapors travel up and out of the quart can and proceed to disperse down the sides of the gallon can before rotating up and out of the container. This would idicate a greater quanity of material occupying the gallon can headspace rather than the quart can's. This phenomena may be due to the close proximity of the quart can surface to the solid 2, 4-DNT compared to the gallon can; if the can was disturbed while equilibrating some trace amount of material could have contacted the container wall. Additional uncertainties may arise with environmental fluctions; the humidity and temperature of the testing space may fluctuate on a day-to-day basis resulting in an increase or decrease in vapor emission. Finally, the loss of surface area were the quart can and sniffer tin were placed could also have affected the amount of residue extracted.

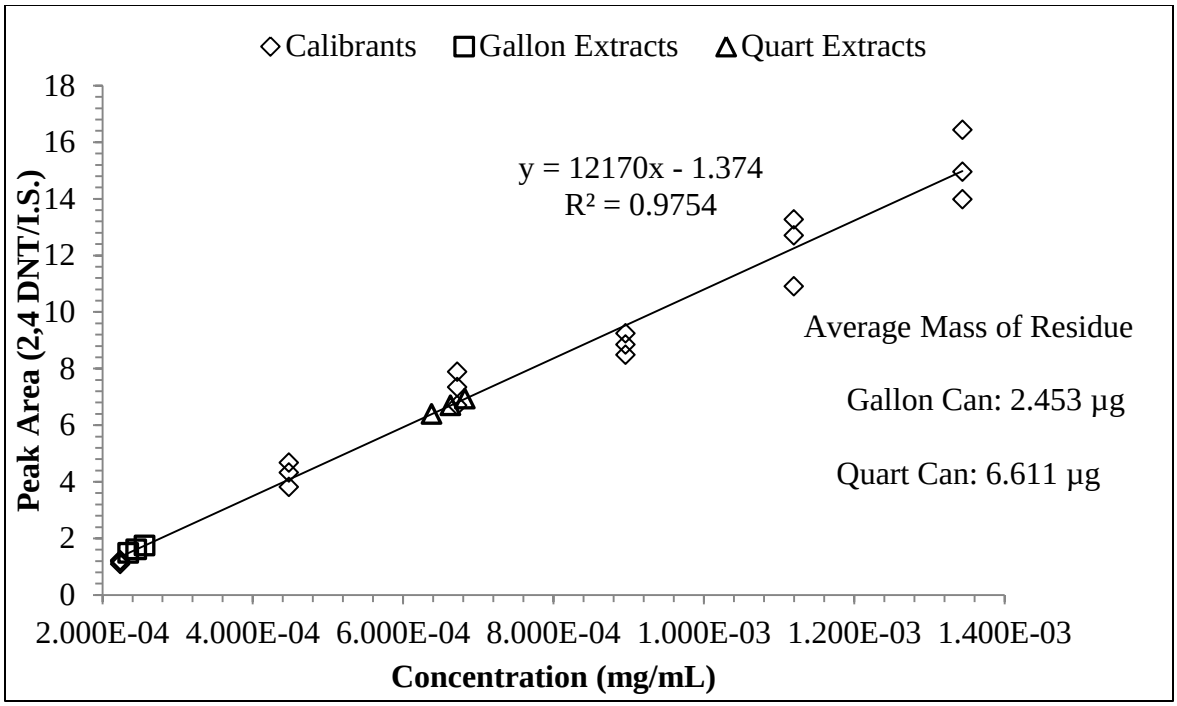


Figure 4.7: Quantitation of 2, 4 DNT Residues Extracted from Containers.

4.4. Conclusions

4.4.1. The Nitrate Hypothesis

Although it was suggesed that nitrogen oxides are formed from decomposing nitric acid in nitrated explosives and thus may be the source of canine alerts, nitrates were not reliably detected in any of the nitroaromatic explosives tested. While Composition C-4 and TNT did produce slightly acidic solutions (see Table 4.1), the formation of nitric acid was not indicated in subsequent testing using LC/MS or the diphenylamine test. The acidity of the solutions are likely cause by impurites, signature compounds, or ERC's within the explosive mixture or by the de-protonation of the nitroaromatic ring. To test the validity of this statement, additional pH measurements were taken for compounds associated with TNT and Composition C-4 (see Table 4.2). Each of the odor compounds

tested exhibited an acidic pH. The measured pH of 2, 4 DNT compared to that of TNT was slightly higher however upon comparing the pKa value of TNT (~15) to that of 2, 4-DNT and taking into account the standard deviations of each, the difference can be attributed to measurement error. The acidic nature of Composition C-4 may also be attributed to the cyclohexanone present in the mixture with a lower pH than that of the base explosive.

Table 4.2: Measured pH of Suspected Odor Compounds.

Compound	Application	Average pH			pKa
2, 4 DNT	ERC	6.56	+/-	0.061	13.75
2-ethyl-1-hexanol	Signature Compound	5.53	+/-	0.029	10.18
Dioctyladipate (DOA)	Plasticizer	5.65	+/-	0.039	N/A
DMNB	Taggant	6.07	+/-	0.009	N/A
Cyclohexanone	Signature Compound	4.54	+/-	0.012	~15

Because nitrate has been eliminated as a source of canine alert to nitroaromatic explosives, various possibilities warrant exploration. First, 2, 4-DNT is a recognized impurity within TNT. Studies have shown a significant 2, 4-DNT presence in the headspace of TNT and have suggested this compound as a possible cause of canine alert to TNT. The possibility of 2, 4-DNT as an odor compound of TNT will be explored in Chapter 5.

Also, according to Table 4.1, Composition C-4, a plastic bound explosive, yielded the most acidic solution. This introduces the possibility that Composition C-4 odor compounds are generated by the plasticizers themselves over time as they react and absorb atmospheric water. For example, a common plasticizer in Composition C-4 is diocyladipate (DOA). The reaction of DOA with atmospheric water results in the

formation of 2-ethyl-1-hexanol, a volatile compound whose presence is seen in the headspace of Composition C-4. Other compounds found in the headspace include cyclohexanol, a solvent in RDX manufacture, and 2,3-dimethyl-2,3-dinitrobutane (DMNB), a taggant. Because Composition C-4 is comprised of several volatile compounds, each of which could be cause of canine alert, live canine studies were conducted on each compound found in the headspace and the results shared in Chapter 5.

4.4.2 Extraction of 2,4 DNT Residues

A method for the detection and separation of TNT and DNT isomers by GC/MS was developed. The purpose of this method was first, to determine what amount of 2, 4 - DNT vapor would rise and adhere to the inside of a container wall, and second, to determine if the amount of vapor lost to surface adhersion was significant enough to effect canine detection. This amount was determined to be ~2.5 µg in a gallon sized can and 6.6 µg in a quart sized can. The amount of explosive expected to saturate the headspace of the container was also determined using the Ideal Gas Law. The amount of material adhering to container walls greatly exceeded the amount expected in the headspace indicating a significant loss of material to 2, 4-DNT surface adhesion. This can be attributed to the “sticky” nature of the 2, 4-DNT compound and suggests a terndency for 2, 4-DNT to revert back to its solid phase after emission from the solid material. Depending on the amount of explosive present, canine detection could be greatly affected.

Specifically, when testing and training canine to detect trace amounts of explosive, the effects of surface adhesion, and thus loss of material, must be accounted for if one wishes to obtain accurate results.

CHAPTER 5. CANINE TESTING

5.1. Introduction

While canines have long been regarded as one of the most reliable and sensitive means for the detection of explosives, the means by which this is accomplished are not well understood. The purpose of this study was to determine the effects of odor availability and test theories presented earlier in this work using certified explosives-detecting canines. In addition it was our goal to identify the compounds within the explosive mixtures TNT and Composition C-4 that are the cause of canine alerts to these explosives.

5.1.1. Odor Availability

Once again, it is a common misconception that the amount of explosive vapor available for canine sampling is determined by the amount of explosive present. Several studies, however, have been conducted that have derailed this misconception. For example, one study on canine detection of landmines noted the influences of soil type,

moisture, and climatic changes on canine detection success [35]. Another study evaluated the effect of strenuous physical activity and found a decreased detection success in "panting" canines [36]. Published accounts regarding canine sensitivity, accuracy, selectivity, and memory also exist. While each of these studies have proven paramount to the development of effective testing and training protocols thus far, studies of the response of canines as a function of odor availability have not been conducted.

It has been shown, in this work and others, that the amount of explosive vapor emanating from an explosive compound may be dictated by several factors including vapor pressure, diffusion coefficient, mass transfer, temperature, sample amount, and the degree of confinement. In this study several theories have been explored and models describing the behavior of explosives in various conditions have been introduced. The purpose of this phase of the study is to test the theories introduced thus far using certified explosives-detecting canines. In particular, the effects of sample volume and degree of confinement on the detection of nitromethane will be evaluated, two factors which have not been explicitly controlled in the past.

5.1.2. Odor Compounds

The vapors emanating from an explosive are often complex thus identification of the odor compounds responsible for triggering a canine response is difficult. Odor discrimination studies have employed several approaches. Some have involved placing explosives and distractors in containers and evaluating canine response [16, 25]. Another approach was to train the canine on a single explosive (such as Composition C-4) then

monitor the canine's response to variations of the explosive mixture [37]. Additionally, suspected odor compounds have been isolated using SPME measurements where compounds were identified in the headspace of various explosives [2, 33, 38].

Compounds identified include nitrobenzene (DNB) and dinitrotoluene (DNT) for TNT, diphenylamine, ethyl centralite, nitroglycerine for smokeless powders, and 2,3-dimethyl-2,3-dinitrobutane (DMNB), cyclohexanone, dioctyladipate (DOA), and its degradation product 2-ethyl-1-hexanol for Composition C-4. These "suspects" were then tested using certified canines; unfortunately, response to suspected odor compounds was generally low with the exception of cyclohexanone and 2-ethyl-1-hexanol, who exhibited small responses. Others studies have identified potential odor compounds, however most of these were unrelated to the explosive itself.

In Chapter 4 of this work several potential odor compounds were explored and identified using instrumental analysis. These compounds include 2, 4-DNT for TNT and cyclohexanone, DMNB, DOA, and 2-ethyl-1-hexanol for Composition C-4. The purpose of this portion of the study was to test the identified odor compounds using certified explosives-detecting canines.

5.2. Materials and Methods

5.2.1. Odor Availability

A preliminary test was conducted in the winter of 2010 in Indianapolis, IN using three explosives-detecting canines. This study used 12 cans setup according to Figure 3.2 where a 2 oz. sniffer tin (Specialty Bottle, Seattle, WA) is placed within a quart sized can that is then placed inside a gallon sized can. The effect of confinement was studied using sniffer tins with a 1/8 inch hole and an asterisk patterned perforation (see Figure 3.1). The effect of sample amount was tested by placing 0.1 mL and 1 mL volumes of nitromethane (Sigma Aldrich, St. Louis, MO) in each of the sniffer tins.

The cans were arranged in a circle with 4 “hot cans”, cans containing the actual explosive; the remaining cans contained water (see Figure 5.1). Each canine was allowed two passes around the circle and positive alerts were rewarded 100% of the time.

		CONFINEMENT	
		1/8-inch hole	Star pattern
AMOUNT	0.1 mL	1 nitromethane 2 water	1 nitromethane 2 water
	1 mL	1 nitromethane 2 water	1 nitromethane 2 water

Figure 5.1: Can Arrangement in Odor Availability Canine Trials.

Based on the results of preliminary testing a larger follow-up study was conducted in Forte Mead, MD using thirty-three certified canine-handler teams from eight different agencies (see Table 5.1). These tests employed the same setup and protocol as before, however, the sample volumes were increased to 1 mL and 10 mL quantities.

Table 5.1: List of Canine Trial Participants.

AGENCY	# OF PARTICIPANTS
Department of Defense (DOD)	5
Federal Protection Service (Homeland Security)	12
Naval Surface Warfare (NW)	1
Marines	4
Amtrak	4
Capital Police	4
Air Force	1
Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF)	2

5.2.2. Odor Compounds

This study was aimed at testing several odor compounds of the explosives TNT and Composition C-4. The compounds tested were 2, 4-DNT, cylcohexanone, DMNB, and 2-ethyl-1-hexanol purchased from Sigma Aldrich (St. Louis, MO). Also tested was the plasticizer DOA purchased from Fisher Scientific Company (Hanover Park, IL). Explosives were provided by Rick Strobel with the Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF). The training aids TNT-NESTT, RDX-NEST, and a Silica Distractor were also tested and provided by Dudley Salmon with the ATF.

This experiment was organized in the same manner as the odor availability study with a few minor exceptions. First, each explosive, odor compound, and distractor was placed in a sniffer tin with an asterisk pattern. The cans were arranged in a circle of 15 with TNT and Composition C-4 each in its own circle. Each circle had 2 “hot cans” with the remaining cans containing the odor compounds and distracters (paperclips, water, eraser, etc.). Figure 5.2 shows the experimental design.

		ODOR TYPE		
		Target Odors	Test Odors	Distractors
REWARD SCHEDULE	75%	Composition C-4 RDX-NESST	-	-
		TNT TNT-NESST		-
	No Reward	-	2-ethyl-1-hexanol Cyclohexanone DMNB	10 distractors
			2,4-DNT	12 distractors

Figure 5.2: Can Arrangement in Odor Compound Canine Trials. Target odors are those odors on which the canine has been imprinted. Test odors are those that have been suggested as the cause of canine alert.

Each dog was allowed two passes resulting in a total of two exposures to the test and target compounds. This study employed the use of an intermittent reward system which rewards a positive alert to the target compounds 75% of the time; a canine alert to a test compound was not awarded. This intermittent reward system is advantageous because it will eliminate the possibility of the canine unintentionally imprinting on the sample of interest and affecting the integrity of the results. This will also avoid the reinforcement of false alerts.

A preliminary test was performed in Indianapolis, IN using three certified canines. This was followed up with a larger study conducted in Forte Mead, MD using the same experimental design. All explosives and test compounds were studied in 10 g or 10 mL quantities.

5.3. Results and Discussion

5.3.1. Odor Availability

The results of the preliminary canine testing are shown below in Table 5.2

Table 5.2: Results of Preliminary Odor Availability Trials.

SAMPLE VOLUME	$1/8^{th}$ INCH HOLE	ASTERISK PATTERN
0.1 mL	8/16	10/16
1 mL	14/16	15/16

The most notable result is the significantly decreased response to the smaller amounts of nitromethane. This directly contradicts the theory presented earlier in this work stating that the amount of explosive present will not greatly affect canine detection success. However, when previous headspace studies are taken into account, this result is not so surprising. An earlier study was conducted using simple headspace sampling that found the amount of explosive liquid needed to sufficiently saturate a container. This study found that the amount of nitromethane needed to fully saturate a one gallon container is approximately 0.306 mL. [39]. This effect is seen in this canine study, because the volume of 0.1 mL was insufficient to saturate the headspace of the one gallon can, the decreased response would be expected.

Because it was found the 0.1 mL was insufficient to fully saturate the gallon can, the sample volumes were increased to 1 and 10 mL quantities and tested in a larger follow-up study. The results of these trials are shown in Figure 5.3 where error bars show the 90% confidence interval around the mean. As expected, the response was largely flat

across all categories, showing no significant change in canine detection success. While there is an apparent increased response to the 1 mL volume in the more confined system, this was deemed statistically insignificant.

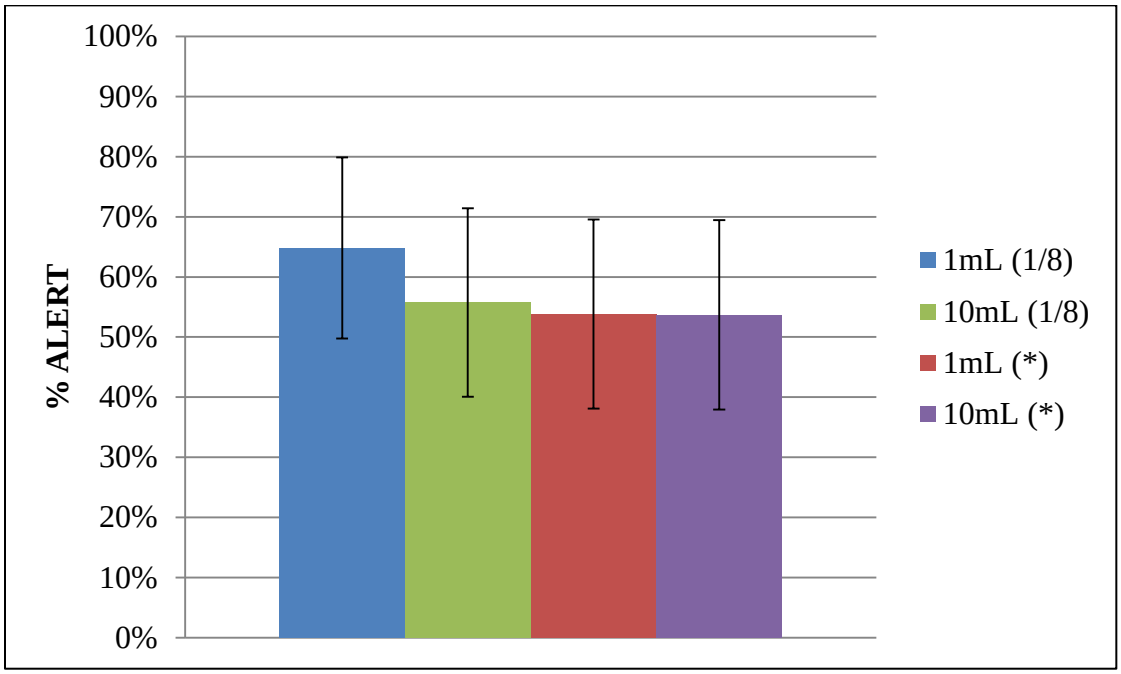


Figure 5.3: Effect of Sample Volume and Confinement on Canine Detection Success of Nitromethane. This includes only those canines who exhibited at least one positive response to the explosive.

5.3.2. Odor Compounds

The preliminary canine trials focused on recognized potential odor compounds. The compounds tested were DOA, 2-ethyl-1-hexanol, and dioctlysebacate (DOS), a plasticizer associated with Composition C-4 and 2, 4-DNT and 3, 4-DNT associated with TNT. Also included were TNT-NESTT and RDX-NESTT in their respective trials. Results of these preliminary trials are shown in Tables 5.3 and 5.4. In these trials none of the canines alerted to RDX-NESTT while 1 canine alerted to both TNT-NESTT and 2, 4-DNT.

Table 5.3: Results of Preliminary Composition C-4 Canine Trials.

Dog	Sniffs	Alerts to C-4	False Positives	Accuracy for C-4
Molly	4	3	0	75%
Radar	4	0	0	0%
Elvis	4	4	0	100%

Table 5.4: Results of Preliminary TNT Canine Trials.

Dog	Sniffs	Alerts to TNT	False Positives	Accuracy for TNT
Molly	4	3	1	75%
Radar	4	1	0	25%
Elvis	4	3	2	75%

The results of the larger follow-up study are shown in Figure 5.3. As expected those canines that alerted to TNT (either in pure form or in NESTT form), had a significantly increased response to 2, 4-DNT. This result was expected as 2, 4-DNT is a known impurity in TNT and is present in the headspace of TNT with approximately 100 times more abundance. The error bars in Figure 5.4 were calculated at the 90%

confidence interval. Because this study only used trace quantities (~10 g) of material, the possibility of the canine detection limit not corresponding to these sample amounts is also possible and should be explored in future work.

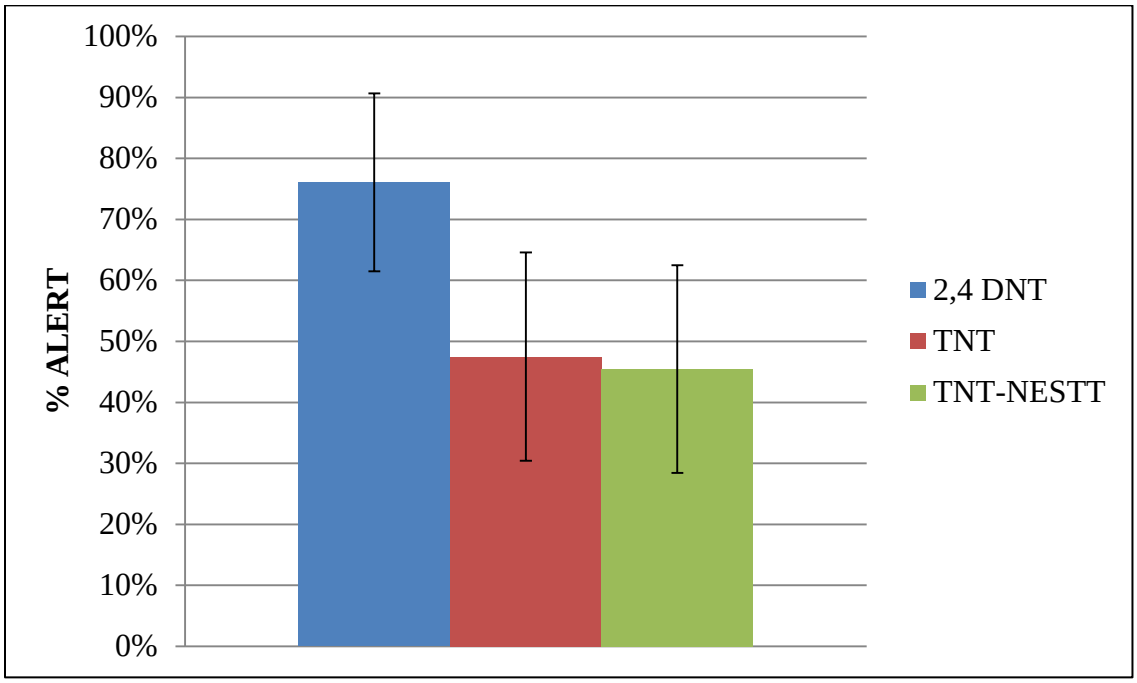


Figure 5.4: Results of TNT Canine Trials.

Finally, the response of canines to Composition C-4 and its related odor compounds was measured. In these trials, the majority of canines alerted to the Composition C-4 with a higher frequency than any other compound tested. Surprisingly no significant response to DOA or its degradation product 2-ethyl-1-hexanol was seen. Furthermore, none of the canines alerted to the training aid RDX-NESTT. There was a notable response to the solvent cylcohexanone and the taggant DMNB, however (see Figure 5.5).

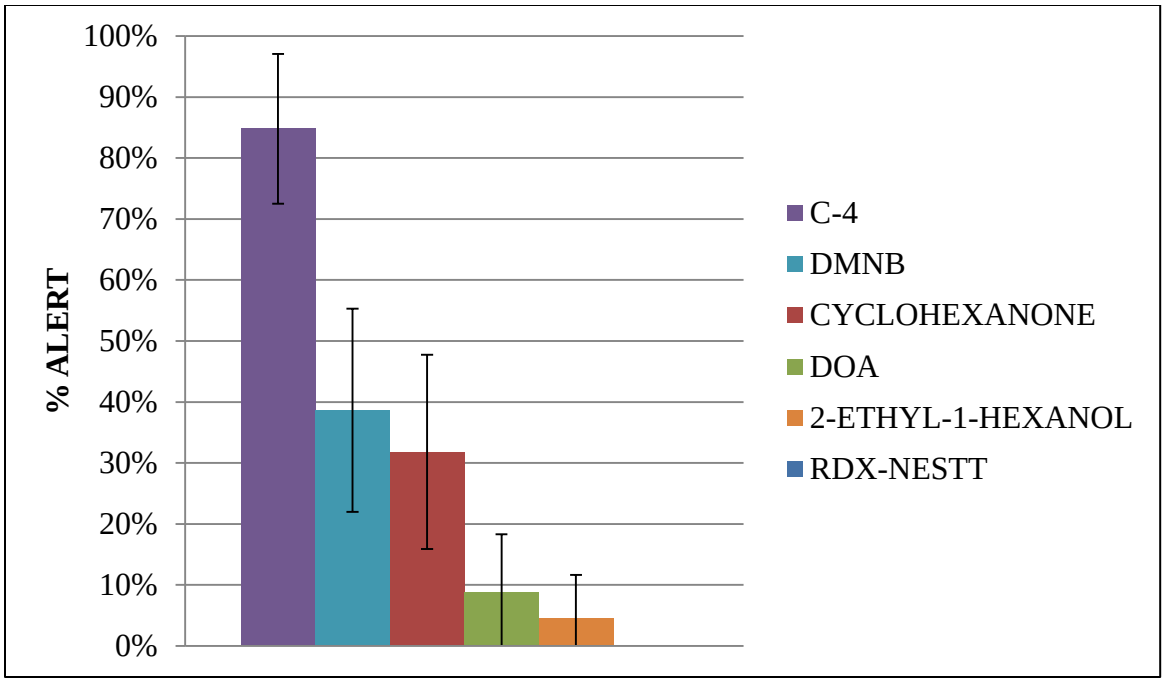


Figure 5.5: Results of Composition C-4 Canine Trials.

5.4. Conclusion

5.4.1. Odor Availability

The theories introduced throughout this work were validated and supported using certified explosives-detecting canines. Overall it was determined that the sample volume and degree of confinement would not impact canine detection success so long as the volume present is sufficient to fully saturate the container in which it is confined.

5.4.2. Odor Compounds

The purpose of these experiments was to isolate and identify the compounds responsible for canine alerts to TNT and Composition C-4. These results indicated that for the explosive TNT, canines exhibited a strong preference for 2, 4-DNT, which is a known impurity and odor compound of TNT. Because GC/MS studies have shown 2, 4-DNT to be present in the headspace of TNT with significantly more abundance, this result in not unexpected. With this knowledge, the development of more effective testing and training protocols is possible.

In the case of the plastic explosive Composition C-4, several compounds within the mixture were tested, including a solvent, plasticizer, and taggant. While a slight response to the solvent cyclohexanone and the taggant DMNB was seen, the explosive itself (Composition C-4) was by far a more attractive target for all of the canines tested.

The results of this study showed an apparent preference for odor generated from the explosive mixture itself rather than a single odor from one particular compound. This too will aid in developing more effective protocols for imprinting and testing canines sensitivity to the explosive Composition C-4.

CHAPTER 6. RECOMMENDATIONS

6.1. Modifications and Future Work

6.1.1. Odor Availability

The odor availability of an explosive is governed by several factors including temperature, degree of confinement, diffusion coefficient, and vapor pressure. The evaporative behavior of explosive liquids was evaluated with the goal of developing a mathematical model describing such behavior. While a model was determined based on Deegan's Model for the evaporation of a spherical drop on a flat surface, because ultimate control of experimental and chemical conditions was not possible, the results showed only qualitative agreement between theoretical and experimental values.

There are several areas of improvement necessary for the successful development of such a model to occur. Upon determination of theoretical values, accurate temperature and humidity measurements were taken, however, convection currents within the test chamber were not accounted for. Additionally, surface tension limitations of the heavier nitroalkanes did not permit measurement on a flat surface, a parameter which was specified by the Deegan Model. If a more accurate determination and model is to be developed, strict control of experimental conditions should occur (*i.e*. temperature, humidity, convection currents). Finally, because only nitroalkanes were tested, additional trials should be conducted on various explosives and their related odor compounds (*i.e*. cylcohexanone, ethyl centralite, DOA, DOS, etc.). Mass loss measurements have been collected for some of these compounds; however extended analysis using ProAnalyst software is needed.

The determination of flow rates of larger volumes (10 mL) of nitrated explosives proved difficult, with actual values being slower than predicted. This was due to the larger sample volume and limited container space; the 10 mL volumes filled the bottom of the container completely, thus resulting in a liquid pool rather than a sphere. It is for this reason that a model describing the mass loss of a liquid pool is needed. Development of such a model should start with Barry's model [40] . Using the Stiver-MacKay method, Barry's model describes the evaporation of a liquid pool in still air (see Equation 6.1)

$$\boldsymbol{E = k\frac{PM}{RT}} \quad \text{(Equation 6.1)}$$

In this model, *E*, defined as evaporative flux, is assumed to be independent of the pool geometry. The effects of free and forced convection are also accounted for by the inclusion of the mass transfer coefficient (*k*). Finally, *P*, *M*, *R*, and *T* account for vapor pressure, molecular weight, gas constant, and absolute temperature. The validity of this model should then be tested experimentally using larger sample volumes under controlled conditions.

Flux measurements were taken to determine the behavior of explosive liquids under confined conditions. These measurements showed the flow rate to be proportional to the hole diameter while flux showed a linear dependence on the number of perforations. These results were visualized using dry ice where the movement of material could be seen. While this will aid in our understanding of explosives behavior, evaluation of behavior under realistic circumstances is necessary. For example, because explosives are often concealed in containers such as backpacks, vehicle trucks, and suitcases, the dry ice experiment should be expanded to include these container types. This would allow examiners to visualize vapor movement under realistic conditions. Finally, such a study could also be extended to include evaluation of the effects of governing factors such as temperature.

The odor availability studies conducted primarily focused on nitrated explosive liquids in pure form. However, in real-world circumstances, acquisition of explosives without impurities is unlikely. The odor availability studies evaluated the evaporation of the explosive Kinestik and a nitromethane/nitroethane mixture and showed a slightly slower mass loss rate as purity decreased. Despite these findings, more research is needed to determine the effect of purity on the evaporation of additional explosive mixtures.

6.1.2. Odor Compounds

Although nitric acid was eliminated as a cause of canine alert to nitroaromatic explosives, the possibility of nitric as a potential odor compound of other nitrated explosives (nitrocellulose, nitroglycerine) should also be explored due to the formation of nitric acid by nitrate esters. In these studies, 2, 4-DNT was determined to be the cause of canine alert to TNT; this was determined instrumentally and using certified explosives-detecting canines. Furthermore, live canine trials determined that no one compound in particular was the cause of canine alert to Composition C-4. Instead there was a strong preference for the explosive mixture as whole.

This study was extended to include analysis of 2, 4-DNT residues on the side of container walls. Using GC/MS the amount of explosive residue adhering to container walls was determined. It was concluded that the amount of residue extracted off of the container walls was significant compared to the amount of vapor expected in the headspace of the container. To say with the certainty that the above is true, a method for the detection of 2, 4-DNT in the headspace of the container should be developed. This headspace study would utilize a standard solid-phase microextraction (SPME) method coupled with GC/MS [33].

SPME offers several advantages for the detection of volatiles. A silica coated fiber is chosen based on the characteristics of the analytes studied; published studies have used a variety of fibers for explosives analysis [33, 39, 41]. The fiber is then exposed to the headspace above the compound or mixture where upon a series of liquid to gas phase transports occur. The gas phase analytes are then collected onto the surface of the SPME

fiber where upon subsequent extraction will occur. Headspace measurements can be taken by using a specialized apparatus (MPS 2) which allows for the direct measurement of the headspace within a gallon or quart sized container.

The above study should also be expanded to include additional explosives. Of particular interest are plastic-bound explosives whose materials are less volatile and "sticky" in nature. This study would also use a SPME-GC/MS method [41] for detection of chemical signatures within plastic-bound explosives. SPME-GC/MS studies on Composition C-4 based explosives (Detasheet, Shape Charge) have occurred [39] and identified solvents (cyclohexanone) and a taggant (DMNB) in the headspace, however successful detection of PETN did not occur. A method for the evaluation of PETN and its explosives such as Semtex and Primacord should be conducted to determine its potential odor compounds. A method for the detection of PETN in a detonation cord using SPME-GC/MS has been reported [42]. SPME studies to identify potential odor compounds of PETN should be conducted and validated using explosives-detecting canines.

6.1.3. Canine Testing

The theories introduced throughout this work were tested using certified explosives-detecting canines. The results of the nitromethane study included only those canines who successfully alerted to nitromethane at least once. This resulted in 27 total alerts to evaluate; several false positives were observed for some of the trial participants. This may be due to nitro methane's significant volatility; this in conjunction with the

small room size may have resulted in total saturation of the room where testing occurred. Because testing occurred over several hours each day this effect is likely and would cause canine confusion in the trials occurring later in the day.

A study of the plume of nitromethane should be conducted to determine what effect this may have on the canines' ability to successfully alert to this compound. Environmental studies of gas plumes have been conducted [43]. One study reported the detection of gases emitted from a ship using an Aerodyne aerosol mass spectrometer (AMS) [44] while another reported the development of a model to describe gas simulations and dispersion [45]. If the rate of expansion of a nitromethane plume was determined, one could determine the amount of time it would take for a room to become saturated with vapor based on sample volume and temperature.

Odor compound trials were conducted using certified explosive-detecting canines to determine the cause of canine alerts to Composition C-4 and TNT. The Composition C-4 studies were largely successful with the majority of canines tested alerting positively to this explosive. The same canines, however, showed great difficulty when attempting to detect TNT. There could be several causes for this difficulty. First, the study used flake TNT; it is possible that TNT in flake form may have some sort of coating on it which would inhibit vapor generation. Second, this study was conducted in an unheated building in March where the temperature varied from 35-40 ºC, therefore it is probable that the low temperature decreased the vapor generation potential of the TNT.

A final factor to consider is the training of each canine. Because several agencies were included in this study, several training regimens should be considered. Canine training protocols differ between agencies when factors such as the explosive type they

are imprinted on, what quantity of explosive is used for training, the container in which the explosive is confined, and the reward system are considered. This study focused on trace detection of explosives (~10 g) and used containers designed by the ATF and used in the National Odor Recognition Test (NORT). Many of the canines tested were not adapted to these container types or sample amounts thus difficulties may have arisen and skewed the results. In future testing, a detailed description of the canines' training should be collected to account for any discrepancies during testing.

6.2. Additional Interests

In recent years, significant strides have been taken in the field of explosives research and canine detection, however several areas of uncertainty still exist. Much more research is necessary to fully optimize canine training and testing protocols. Continued research into the development of "electronic noses" is of interest. Several studies have been reported for the detection of explosive vapors using various techniques. A method for the rapid detection of explosives and illicit drugs using a pre-concentration device inputted into an ion mobility spectrometer, has been developed [46]. A field deployable sniffer for 2, 4-DNT detection has also been reported [28]. This device is an expansion of existing artificial nose technology and uses an optical fiber bundle with sensors to mimic the canine nose. Research into the development of an artificial olfactory system is also underway [47]. This research will account for such factors as detection limits, analysis speed, and portability.

Additional insight into the use of canine training aids is also of interest. The canine trials conducted in this study showed TNT-NESTT to be an effective training aid for TNT, with more canines alerting to this compound than the actual explosive. The Composition C-4 training aid, RDX-NESST, on the other hand, did not illicit any response from any of the subjects tested. Evaluation of additional training aids, such as Scentlogix, is necessary. These studies would include SPME and/or headspace measurements, pH measurements, and finally canine detection evaluation in a controlled study.

The evaluation of canine detection procedures as well as the instrumental analysis of the explosives included in this study will aid in the developmental of more effective canine testing and training protocols. Chemical analysis will aid in our understanding of the explosive materials under various conditions and in the future increase the success of detection prior to denotation. Additional research is necessary and will further aid in the fight against terrorism.

LIST OF REFERENCES

LIST OF REFERENCES

1. Settles, G.S., *Sniffers: Fluid Dynamic Sampling for Olfactory Trace Detection in Nature and Homeland Security- The 2004 Freeman Schlor Lecture.* Journal of Fluids Engineering, 2004. **127**(2): p. 187-218.
2. Furton, K.G., and L. J. Myers, *The scientific foundation and efficacy of the use of canines as chemical detectors for explosives.* Talanta, 2001. **54**: p. 487-500.
3. Correa, J.E., *The Dogs Sense of Smell*. 2011, Alabama A&M and Auburn Universities.
4. Firestein, S., *How the olfactory system makes sense of scent.* Nature, 2001. **413**: p. 211-218.
5. Ewing, R.G.a.C.J.M., *Detection of Volatile Vapors Emitted from Explosives with a Handheld Ion Mobility Spectrometer.* Field Analytical Chemistry and Technology, 2001. **5**(5): p. 215-221.
6. J.W. Gardner, P.N.B., *Electronic Noses-Principles and Applications.* Measurement Science and Technology, 2000. **11**(7): p. 1087.
7. Li, J., *The Cyranose Chemical Vapor Analyzer.* Sensors, 2000. **17**(8): p. 56-60.
8. Yinon, J., *Detection of Explosives by Electronic Noses.* Analytical Chemistry, 2003. **75**(5): p. 98-105.
9. Steinfeld, J.I.a.J.W., *Explosives Detection: A Challenge for Physical Chemistry.* Annual Review fo Physical Chemistry, 1998. **49**: p. 203-232.
10. Krausa, M., ed. *Vapor Dectection of Explosives for Couter-Terrorism* Vapour and Trace Detection of Explosives for Anit-Terrorism Purposes, ed. M.K.a.A.A. Reznev. 2004, Kluwer Academic Publishers: Boston. 1-9.
11. Dionne, B.C., et al., *Vapor Pressure of Explosives* Journal of Energetic Materials 1986. **4**: p. 447-472.
12. Bender, E., et al., *Surface Contamination by TNT.* Journal of Forensic Science, 1992. **37**(6): p. 1673-1678.
13. Engel, T., *Thermodynamics, Statistical Thermodynamics, and Kinetics*. Second Edition ed. 2006: Pearson Prentice Hall. 1.
14. Green, D.W.a.R.H.P., *Heat and Mass Transfer*, in *Perry's Chemcial and Engineer's Handbook 8th Edition*.
15. Singh, S., *Sensors-An effective approach for the detection of explosives.* Journal of Hazardous Materials, 2007. **144**: p. 15-28.
16. Strobel, R., R. Noll, and J. Kury. *Nitromethane K-9 Detection Limit*. in *7th International Symposium on Analysis and Detection of Explosives* 2001. Edinburgh, Scotland.
17. Deegan, R.D., *Pattern formation in drying drops.* Physical Review, 2000. **61**(1): p. 475-485.

18. Deegan, R.D., et al. , *Contact line deposits in an evaporating drop* Physical Review 2000. **62**(1): p. 756-765.
19. Dunn, G.J.e.a., *The Effect of the Thermal Conductivity of the Substrate on Droplet Evaporation.* Journal of Fluid Mechanics, 2009. **623**: p. 329-351.
20. Fuller, E.N., P.D. Schettler, adn C.J. Giddings, *A New Method fro Prediction of Binary Gas-Phase Diffusion Coefficients.* Industrial and Engineering Chemistry, 1966. **58**: p. 19-27.
21. Strobel, R.A., R. Noll, and C.R. Midkiff, *The ATF Canine Explosives Detection Program*, in *4th International Symposium on Analysis and Detection of Explosives*, J. Yinon, Editor. 1993, Kluwer Academic Publishers: Jerusalem, Israel. p. 455-461.
22. Agency, E.P., *EPA Online Tools for Site Assessment: Estimated Diffusion Coefficient in and Water-Extended Version*. 2010.
23. Lide, D.R., *Handbook of Chemistry and Physics*. 1995, Boca Raton, FL: CRC Press.
24. Davidson, W., et al., in *Sensors, and Command, Control, Communications, and Intellegance Technologies for Homeland Security and Homeland Defense III*. 1994. p. 108-119.
25. Strobel, R.A., R. Noll, and C.R. Midkiff. *The ATF Canine Explosives Detection Program* in *4th International Symposium on Analysis and Detection of Explosives*. 1993. Jerusalem, Israel: Kluwer Academic Publishers
26. Bruno, T.J.a.J.W., *Gas Saturation Vapor Pressure Measurements of Mononitrotoluene Isomers from (283.25-313.15)K.* Journal of Chemical Engineering Data, 2010. **55**: p. 159-164.
27. Oxley, J.C., et al., *Quantification and Aging of the Post-Blast Residue of TNT Landmines.* Journal of Forensic Science, 2003. **48**(4): p. 742-753.
28. Albert, K.J., et al. , *Field Deployable Sniffer for 2,4-Dinitrotoluene Detection.* Environmental Science Technology, 2001. **35**: p. 3193-3200.
29. Silberrad, O.a.F., R. , *The decomposition of nitrocellulose.* Journal of the Chemical Society, Faraday Transactions, 1906. **89**: p. 1182-1186
30. Bender, E.C., *Analysis of Low Explosives*, in *Forensic Investigations of Explosions*, A. Beveridge, Editor. 1998, Taylor and Francis: Bristol, PA. p. 343-388.
31. Cummins, J., et al., *Separation and identification of anions using porous graphitic carbon and electrospray ionization mass spectrometry: Application to inorganic explosives and their post blast residues.* Analytical Methods, 2011. **3**(7): p. 1682-1687.
32. Hubshmann, H.-J., *Handbook of GC/MS: Fundamentals and Applications*. Second ed. 2009, Weinheim: WILEY-VCH.
33. Harper, R.J., J.R. Almirall, and K.G. Furton, *Identifation of dominant odor chemicals emanating from explosives fro use in developing optimal training aid combinations and mimics for canine detection* Talanta, 2005. **67**: p. 313-327.
34. Williams, S. (2008) *Stock solution for preparation of diphenylamine reagent for cellulose nitrate identification*. Conservation DistList Instance.

35. Goth, A., McLean, I., Travelyan, J, *How do dogs detect landmines?* , in *Mine Detection Dogs: Training, Operations, and Odour Detection*. 2003, Geneva International Centre for Humanitarian Demining: Geneva, Switzerland.
36. Gazit, I., Terkel, J. , *Explosives detection by sniffer dogs following strenuous physical activity* Applied Animal Behavior Science, 2003. **81**: p. 149-161.
37. Dixon, S.R., et al. , *Analysis of Explosive Vapour Emission to Guide Development of Vapour Detectors*, in *NATO Advanced Research Workshop on Vapour and Trace Detection of Explosives for Anti-Terrorism Purposes*. 2003, Kluwer Academic Publishers: Moscow, Russia.
38. Lorenzo, N., et al. , *Laboratory and field experiments used to identify Canis lupus var. familiaris active odor signature chemicals from drugs, explosives, and humans.* Analytical and Bioanalytical Chemistry, 2003. **376**: p. 1212-1224.
39. Lotspeich, E., Kitts, K. Goodpaster, J. , *Headspace Concentratios of Explosive Vapors in Containers Designed for Canine Testing and Training: Theory, Experiement, and Canine Trials*, in *Evaluation of Odor Compounds Sensed by Explosives-Detecting Canines*. 2011, Indiana University Purdue University Indianapolis: Indianaplis.
40. Berry, J., *Estimating Rates of Spreading and Evaporation of Volatile Liquids.* Chemical Engineering Progress, 2005. **101**(1): p. 32-39.
41. Lai, H., Leung, A., Magee, M, Almirall, J., *Identification of volatile chemical signatures from plastic explosvies by SPME-GC/MS and detection by ion mobility spectrometry.* Analytical and Bioanalytical Chemistry, 2010(396): p. 2997-3007.
42. Perr, J., Furton, K., Almirall, J. , *Solid phase microextraction ion mobility spectrometer interface fro explosive and taggant detection* Journal of Separation Science, 2005(28): p. 177-183.
43. Bracken, C., et al. , *An Analysis of Exhaust Emissions from a Large Ship Docked in Humboldt Bay*. 2007. p. pg. 38.
44. Lu, G.e.a., *Identification and characterization of inland ship plumes over Vancouver, BC.* Atmospheric Enviroment, 2006. **40**(15): p. 2767-2782.
45. Sayma, A., Betts, P., *A finite elemental model for the simulation of dense gas dispersion in the atmosphere.* International Journal for Numerical Methods in Fluids, 1998. **24**(3): p. 291-317.
46. Guerra-Diaz, P., Sigalit, G., Almirall, J. , *Dynamic Planare Solid Phase Microextraction-Ion Mobility Spectrometry for Rapid Field Air Sampling and Analysis of Illicit Drugs and Explosives.* Analytical Chemistry, 2010. **82**: p. 2826-2835.
47. Moore, D., *Instrumentation for trace detection of high explosives.* Review of Scientific Instruments, 2004. **75**(8): p. 2499.